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Host Location and Host-associated Divergence In Parasitoids of the Gall Midge, Asteromyia Carbonifera

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HOST LOCATION AND HOST-ASSOCIATED DIVERGENCE IN PARASITOIDS OF THE GALL MIDGE, *ASTEROMYIA CARBONIFERA*

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By

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Jeffrey L. Howell ENTITLED Host location and host-associated divergence in parasitoids of the gall midge, *Asteromyia carbonifera* BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Howell, Jeffrey L. M.S. Department of Biological Sciences, Wright State University, 2016. Host location and host-associated divergence in parasitoids of the gall midge, *Asteromyia carbonifera*.

Some of the world's greatest mysteries are the series of ecological and behavioral processes that promote adaptive radiation: when one species rapidly diverges into multiple descendants due to ecological selective pressures. Selective pressures from natural enemies have the potential to drive such radiations, as has been suggested in the diversification of the goldenrod gall-midge, *Asteromyia carbonifera* (Stireman et al., 2008, 2012). This complex, multitrophic system involves the midge species complex, their goldenrod host plants (*Solidago* sp.), and a suite of parasitoid enemies in the diverse wasp superfamily, Chalcidoidea. There is evidence that the midge is undergoing host-associated differentiation (HAD), in which it is rapidly diversifying into genetically distinct races on different *Solidago* host plants in sympatry (Stireman et al., 2006; 2010). Because the parasitoids may use host plant cues to locate the midges, they may drive midges to shift to new host plants, facilitating population divergence. Subsequently, the parasitoids may eventually colonize the midges on these novel plants and undergo HAD themselves, in a cascading process (Stireman et al., 2006). I used this tritrophic system as a model to understand how interactions between plants, herbivores, and parasitoids drive insect diversification and shape ecological communities.

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The faculty and graduate students of Wright State University's Biological Sciences Department deserve my sincerest gratitude for their constant support over the course of this study. I thank my advisor Dr. John O. Stireman III, as well as my other committee members, Dr. Don F. Cipollini, and Dr. Jeffrey L. Peters for their incredible guidance. I also thank Seth Patrick, Hosanna Loureaux, and Saige Phelps for their contributions to this study as undergraduate research assistants

BACKGROUND

Origins and Structure of Biodiversity

Biodiversity refers to the extraordinary diversity and variety of life on Earth. The different environments, plants, animals, microorganisms, and all of their interactions constitute the vast biodiversity on our planet. Biodiversity helps to stabilize ecosystems, prevents extinctions, inhibits invasive species growth, encourages nutrient cycling, contributes to climate stability, protects water resources, provides food and medicinal resources, and provides the scaffolding for the planet that we live on (Bailey et al., 2009; Duffy, 2009). Understanding Earth's biodiversity and the processes that have given rise to it is the focus of much ecological and evolutionary research.

Most ecosystems are comprised of diverse assemblages of interacting species. These organisms are always interacting with one another in communities, where each organism exerts selective pressure on another as it tries to survive and promote individual reproductive success. Understanding the processes that structure these communities has been a topic of interest for quite some time, and remains an important challenge in ecology today (Ehrlich & Raven, 1964; Bailey et al., 2009). Although these ecological interactions are a consequence of the diversity of species present, they may also be an important underlying driver of diversity itself through the process of ecological speciation (Rundle & Nosil, 2005; Abrahamson et al., 2008; Feder, 2012). Ecological speciation is the process by which barriers to gene flow evolve between populations as a result of ecologically based divergent selection (Rundle & Nosil, 2005). Factors that facilitate speciation can therefore consist of ecological and behavioral events in a particular population, such as a shift between hosts or habitats (Schluter, 2001).

A particularly diverse group of organisms that are fundamental components of most ecological communities are the insects. The estimated 4-6 million species of insects comprise 90% of all animal species, and half of these insects are phytophagous (plant-feeding) (Novotny et al. 2002). Since there are roughly 300,000 plant species on Earth, it is of no surprise that the vast majority of ecological interactions in terrestrial systems occur between plants and their insect herbivores (Poelman et al., 2008). Plant-feeding insect lineages are particularly diverse compared to other functional groups of insects, perhaps as a consequence of intimate interactions with specific host plants (Farrell, 1998). Studies of plant-insect associations are revealing increasing examples of phytophagous insect species that consist of morphologically cryptic yet genetically differentiated populations that are specialized on different plant species. A classic example is seen in the apple-maggot fly, in which populations shifted from hawthorn feeding races onto apples in a relatively short period of evolutionary time (~300 years) (Feder, 1998). The cryptic host-races on apple are both behaviorally and genetically distinct from races on hawthorn (Feder, 1998; Forbes et al., 2009). These ecological speciation events can be explained by the phenomenon of host-associated differentiation (HAD), in which species genetically differentiate due to selective tradeoffs associated with the use of different hosts. (Stireman et al., 2006). These divergent new lineages of phytophagous insects may in turn provide opportunities for new niches to be exploited by predators and parasitoids (Forbes et al., 2009).

Phytophagous insects are often attacked by parasitoids, another highly diverse group of insects. Parasitoids have an intimate and obligate association with their host in that they require them for reproduction, typically laying eggs on or inside of another

insect so that their larvae may feed on the living tissue of the host. Once the larvae have developed, they pupate and eclose as adults, resulting in the death of their host. Among the most diverse of parasitoid taxa are the parasitic hymenopterans. For example, Chalcidoidea comprise a superfamily of tiny parasitic wasps with roughly 22,000 described species and estimates of actual diversity reaching upwards of 500,000 species (Heraty et al., 2013). The mechanism behind this incredible diversity has perplexed ecologists and evolutionary biologists, and the answers may lie in their tight interactions with their hosts much like the phytophagous insects. The intimate relationship between parasitoids and their hosts fosters coevolution, with each player exerting strong selective pressures on one another. As phytophagous insects experience host-associated divergence, parasitoids may follow suit and evolve divergent lineages of their own on new host lineages. The result may be a cascading speciation phenomenon across trophic levels; thus, cascading HAD may provide a mechanism for the astonishing diversity of parasitoids (Stireman et al., 2006; Forbes et al., 2009).

Parasitoid Host Location using Olfactory Cues

Parasitoids may be particularly susceptible to cascading HAD due to their reliance on often highly specific olfactory cues to locate hosts. These tiny parasitic wasps have the difficult task of locating a suitable host in a complex environment and rely on specific stimuli associated with host habitats as detectable and reliable cues during host searching (Egan et al., 2013). The intimate relationship between parasitoids and their hosts allows for coevolution in the form of a perpetual arms race, where parasites are constantly evolving ways to locate and attack hosts while host insects evolve methods of thwarting

or evading parasitoid search and attack efforts. Many parasitoids are capable of detecting subtle olfactory cues and they rely on these cues to assess habitat quality, find food, and find hosts for reproduction (Tentelier & Fauvergue, 2007).These olfactory cues may originate from the host insect or its byproducts; such as frass, cuticular waxes, or saliva (Girling et al., 2011). Additionally, organic volatiles from plants can offer parasitoids detectable and potentially reliable cues to find hosts (Poelman et al., 2008; Tooker et al., 2008).

A reliance on host-plant associated odors may encourage host-associated divergence. Herbivores might be able to obtain a temporary escape ('Enemy Free Space') from their parasitoids by shifting or expanding their host range to new host plants (Godfray, 1994). Behavioral variation in parasitoids that cause attraction to odors on this new host plant-midge interaction may be selectively favored due to lack of competition. Then, spatial or habitat associated mating, along with divergent ecological selection pressures may lead to the formation of plant-associated parasitoid host-races and eventually species.

It is unknown what specific olfactory cues or other stimuli are utilized by most parasitoids during their search efforts. By determining what type of stimuli the parasitoids behaviorally respond to, a better understanding of host location leading to successful parasitism can be achieved. As a result of the narrow specialization of parasitoids on hosts, evolution has likely favored highly specific odor preferences to differentiate between suitable and unsuitable hosts. If parasitoids are differentiating onto different hosts in a cascading HAD process this is likely to be reflected in a highly specific attraction to particular host plants.

The Plant-Midge-Parasitoid Multitrophic System (Research Model)

The multitrophic system I am using as a model to examine olfactory responses and explore the potential for cascading HAD in parasitoids involves host plants, gall midges, a fungal symbiont, and a suite of parasitoid wasps. The goldenrod gall midge, *Asteromyia carbonifera*, forms blister galls on the leaves of goldenrod (*Solidago* sp.) and related host plants with the aid of the fungal symbiont, *Botryosphaeria dothidea* (Heath & Stireman, 2010). Several parasitoid wasps (Eulophidae, Platygastridae, & Torymidae) parasitize the larvae of *A. carbonifera* as they develop inside of the galls (Weis, 1982b).

The close relationships between each species in this trophic system and the apparent sympatric adaptive radiation of the midge across host taxa make this an excellent model system for studying the ecological processes that lead to diversification (Stireman et al. 2006, 2012). Furthermore, a wide range of *Solidago* species are abundant locally in Ohio which allowed me to collect a large number of galls from a variety of host plants.

(i) Asteromyia (Gall Midges)

Many species of gallmaking midges (Diptera: Cecidomyiidae) specialize on goldenrods (Asteraceae: *Solidago spp.*) and related plants in the tribe Astereae as hosts, including *Rhopalomyia solidaginis, Asphondylia solidaginis, Asteromyia euthamiae, Asteromyia carbonifera,* and many other *Asphondylia Rhopalomyia,* and *Dasineura* species (Stireman et al., 2006, 2008 & pers. comm; Dorchin et al., 2015). Genetic and behavioral evidence indicates that *R. solidaginis, Asphondylia solidaginis,* and *A.*

carbonifera have undergone HAD and are adaptively radiating as genetically differentiated lineages on sympatric host plants (Stireman et al., 2006, 2008; Heath & Stireman 2010; Dorchin et al., 2015). In this research I focus primarily on *Asteromyia carbonifera* and their parasitoids.

A. carbonifera is widely distributed across much of North America and attacks goldenrod species in the genus *Solidago.* At least 65 species of *Solidago* have been observed with *A. carbonifera* galls, along with a few related species in the tribe Astereae (Gagné, 1968). These midges usually complete about three generations during the summer season from June to August and form blister galls on the leaves of their host plant (Weis, 1982a; Stireman et al., 2012). The final generation of the season overwinters as mature larvae within the gall, ultimately pupating and eclosing as adults the following spring and summer (Weis, 1983). A somewhat atypical characteristic of these galls is that they are not formed by plant tissue; instead, they are created by a fungal symbiont (*Botryosphaeria dothidea* (Weis et al., 1983; Heath & Stireman, 2010). There is evidence that the fungus is actively transported by the adult female midges in special pockets on the ovipositor, allowing her to deposit both eggs and fungal conidia on the host plant (Borkent & Bissett, 1985; Heath & Stireman, 2010). Midge larvae hatch and induce gall development, growing within a chamber of the gall surrounded by fungal hyphae in a type of mutualistic relationship as the fungus will not proliferate without the presence of midge larvae just as the larvae feed on the fungus and cannot develop without it (Weis, 1983, 1986; Heath & Stireman, 2010).

A particularly interesting consequence of the close relationship between *A. carbonifera* and its fungal symbiont is the formation of morphologically different gall

types. These gall morphotypes can be seen as an extended defensive phenotype of the midge, as the morphologies are likely influenced by selective pressure from parasitoid natural enemies (Stireman et al., 2012). The different gall morphs can occur sympatrically, even on the same ramet or leaf of an individual host plant (Stireman et al., 2008). Recent work by Stireman et al., (2012) has shown evidence that *Asteromyia carbonifera* is rapidly differentiating across host plant taxa and gall phenotype, resulting in cryptic host-associated genetic structure in the midge as a result of ecological interactions between host plant, fungal symbiont, and natural enemies.

(ii) Host plants

The host plants of *Asteroymia carbonifera* are goldenrods within the genus, *Solidago* (Asteraceae). Over 100 species have been described in the genus *Solidago* (Semple & Cook, 2003). These perennial plants often grow in prairies, fields, and forests and are distributed across much of North America. Many species co-occur in sympatric distributions and are valuable components of ecological communities since they host a wide variety of insect herbivores and pollinators (Semple & Cook, 2003). They typically bloom in the summer and are easily recognized by their golden inflorescences with hundreds of small capitulae (Semple & Cook, 2003).

A plethora of ecological studies have been conducted involving plant-insect interactions in *Solidago* (see Weis, 1982, 1983, 1986; Abrahamson et al., 1989; Cain et al., 1991; Root, 1996; Stireman et al., 2005, 2006, 2008, 2010, 2012; Tooker et al., 2008; Heath & Stireman, 2010; Heard et al., 2013), but no previous studies have examined this

system from a tritrophic context to examine host-associated divergence in parasitoids of *A. carbonifera* and their olfactory responses.

My research focuses on the following species due to their local abundance and co-occurrence in Ohio: *Solidago altissima*, *S. gigantea*, *S. patula*, *S. nemoralis*, and *S. juncea*. Some closely related species in the family Asteraceae are also included this study, including *Euthamia graminifolia* and *Symphyotrichum lanceolatum* due to their sympatric range and susceptibility to attack by related *Asteromyia* midges that form blister galls (T. Brown, unpublished data). Furthermore, *Asteromyia euthamiae*, which creates galls on *Euthamia graminifolia*, appear to be attacked by either the same species, or closely related parasitoids to those which attack *A. carbonifera* (T. Brown, unpublished data).

(iii) *Parasitoids*

A suite of hymenopteran parasitoids are known to be natural enemies of *Asteromyia* gall midges. These parasitic wasps hail from the highly diverse wasp superfamilies, Chalcidoidea and Platygastroidea. Chalcidoid taxa that are known to attack *Asteromyia carbonifera* include one torymid, *Torymus capitis*, and several eulophids, including *Baryscapus fumipennis*, *Closterocerus solidaginis*, *Aprostocetus tesserus*, *Aprostocetus homeri*, and an unknown *Aprostocetus* species ("T1") (Weis, 1982a; Stireman et al., 2008). The platygastroid that attacks *A. carbonifera* appears to be *Platygaster solidaginis* (Platygastridae) (Weis, 1982b; Stireman pers. comm). *Platygaster solidaginis* is a gregarious parasitoid of midge eggs or young larvae, the eulophids are likely parasitoids of the larvae, and *Torymus capitis* may demonstrate parasitism of

midge larvae and late-season hyperparasitism of other *Asteromyia* parasitoids (Weis, 1982a, 1983).

Parasitoid wasps have incredibly acute olfactory senses and use odor cues from hosts or host habitats to aid search efforts for a suitable host (Vet et al., 1983; Tentelier & Fauvergue, 2007). These olfactory responses are likely under strong selective pressure, as these wasps are incredibly small (1-2.5mm) and must locate suitable hosts in a complex, three-dimensional environment filled with different olfactory stimuli. Because these parasitoids rely so heavily on olfactory cues from host plants and host to host plant interactions, they would be expected to specialize on hosts on particular host plants, facilitating genetic isolation. Thus, the specialization of these parasitoid populations may allow for genetic divergence along host plant lines.

Objectives

There are three primary questions that I am exploring with the *Solidago*-*Asteromyia*-parasitoid system:

1: Do parasitoids exhibit evidence of host-associated genetic structure similar to their hosts, the *Asteromyia* gall midges? If so, is divergence of parasitoid populations a result of parasitoids evolutionarily following midges onto novel host plants, or are parasitoids diverging relative to plant species and attacking all suitable host midges on those plants

Parasitoid wasps experience heavy selective pressure to develop on or within a suitable host. They must overcome a specific host's physiological defenses in order to develop. Due to this intimate relationship between parasitoid and host, I predict

parasitoids will show evidence of host-associated genetic structure through the existence of genetically distinct host plant-associated clusters as seen in their *Asteromyia* hosts (see Stireman et al., 2006). This evidence of cascading diversification would result from parasitoids following midges evolutionarily as they genetically diverge into different host-associated forms on alternate host plants, resulting in the formation of cryptic species in both the midges and their parasitoids.

If parasitoids show similar patterns of divergence as *A. carbonifera*, then they likely specialize on the specific host plant associated lineage of midge. In the event that no evidence of host-associated genetic structure is seen, then the parasitoids might be midge and host plant generalists, or have diverged so recently that there has been insufficient time for genetic differentiation to occur. Additional support for parasitoids being tied to host plant may be seen if a strong behavioral response to olfactory cues from a specific host plant species is observed.

2: Does the olfactory response of the parasitoids to host plants reflect underlying host plant associated genetic structure?

A series of behavioral olfactory assays will reinforce phylogenetic evidence of host-associated differentiation. First, parasitoids are expected to be attracted to volatiles from *Solidago* or other host plants. Second, parasitoids are expected to be more attracted to odors from their natal host plant species than to other potential *Asteromyia* host plants.

If the olfactory response of parasitoids reflects underlying host plant associated genetic structure, this suggests olfactory stimuli are preferentially selected for during differentiation to optimize searching ability for suitable hosts. If parasitoid olfactory

response does not coincide with host plant origins then they may be attracted to cues from the host larva itself, the fungal symbiont, or other sources.

MATERIALS AND METHODS

Specimen Collection

To obtain parasitoid specimens, *Asteromyia* galls from a range of host plants of varying phylogenetic distances were collected. These host plants include the sister species *Solidago altissima* and *S. gigantea,* other species in the same genus including *S. nemoralis, S. juncea*, and *S. patula,* and two Aster species from different genera, *Euthamia graminifolia* and *Symphyotrichum lanceolatum* (Figure 27, Appendix I). Galls were collected from 13 study sites across Ohio and adjacent states between the months of May and September in 2014. Galls were collected from locations in which at least two different species of goldenrod were growing sympatrically. I collected galls from the following areas in Ohio: Adelphi, Crane Hollow Reserve, Huffman Metro Park, Kiser Lake, Oakes Quarry, Siebenthaler Fen, Creekside Trail in Beavercreek (Factory Road), and private property in Beavercreek and Yellow Springs, Ohio. In addition, I collected galls and plant material along roadsides in Richmond, Kentucky and in New Tazewell and Celina, Tennessee (Table 1, Figure 1). I collected as many leaves with galls that I could find in a particular location with overlapping plant species. These galled leaves were then placed in a plastic Ziploc bag labeled with the collection site, date and plant species from which they came. After a sampling period of about 15 minutes, I would place the bag with plant material in a cooler for the remaining duration of the field sampling period to avoid exposure to direct sunlight. After field sampling, I returned to the lab and individual galls were given a collection label that included site information, date, plant species, and gall morphotype. The galls and labels were then placed into a

glass vial capped with cotton to allow for gas exchange while preventing escape by insects. Vials containing galls were placed in a holding chamber equipped with damp mixture of peat moss and perlite to maintain relatively high ambient humidity levels and prevent the galls from desiccating. Every 3-5 days I replaced water lost from the substrate in these chambers by adding water so that the substrate would remain moist. These storage containers were then stored in an incubator under 16:8 (Light:Dark) hour photoperiod and 28:25°C temperature cycle to simulate natural summer conditions.

Asteromyia galls experience parasitism rates of approximately 30% on average, and thus may contain either midge or parasitoid larvae (Stireman et al., 2008). Due to this uncertainty and non-uniform distribution of parasitoid attack across host plants, it was important to collect many galls from all host plants. I ultimately collected more than 2000 galls from the host plants of interest.

Figure 1. Map of collection sites for parasitoid populations in Ohio, Kentucky, and Tennessee, USA. See Table 1 for full site information.

Table 1. Collection site locations and associated code names.

Collection Site	Code	Latitude	Longitude
Adelphi, OH	ADE	39.46673	-82.747115
Crane Hollow Reserve, Athens, OH	CRN	40.417287	-82.907123
Huffman MetroPark, Dayton, OH	HMP	39.804143	-84.092045
Kiser Lake, St Paris, OH	KIS	40.186003	-83.959873
Oakes Quarry Park, Fairborn, OH	OAK	39.814637	-83.995003
Siebenthaler Fen, Beavercreek, OH	FEN	39.798287	-84.235231
Creekside Reserve, Beavercreek, OH	FAC	39.716392	-84.045157
Beavercreek (Private Property), OH	JBW	39.690236	-84.050929
Great Seal State Park, Chillicothe, OH	GSP	39.39922	-82.949376
Yellow Springs (Private Property), OH	STT	39.806449	-83.886874
Kentucky Wildlife Refuge Area, Richmond, KY	KWRA	37.747857	-84.294654
New Tazewell, TN	NTT	36.442583	-83.599631
Dale Hollow Lake, Celina, TN	DHL	36.550061	-85.505247

Rearing Insects

I checked the vials with galls for eclosion of midges or parasitoids daily. If midges emerged, they were collected with a mouth aspirator and immediately placed into a vial of 75% EtOH along with a label containing the following information: collection site, collection date, eclosion date, host plant, and gall morphotype. If a parasitoid wasp emerged from the gall, it was placed in a 16oz deli cup equipped with a fabric mesh for ventilation and a cotton ball soaked in a 10% honey solution. These cups were given labels with host plant and collection site, and any parasitoids that emerged from the same host plant and site were kept together in these cups to promote assortative mating. These cups were stored in an incubator under 16:8 (Light:Dark) hour photoperiod and 28:25°C temperature cycle.

Behavioral Olfactometry Assays

Host plant preferences of each parasitoid species was determined through behavioral olfactometry assays using a four-choice olfactometer (Fig. 2) as described by Vet el al. (1983). Although it was not generally possible to identify parasitoids to species while they were living, I approached the behavioral testing by attempting to sample as much taxonomic and host plant diversity as possible to obtain data for all species originating from a variety of natal host plants. Air was passed through each olfactometer at a rate of 300ml/min using a flowmeter and all air entering the device was filtered through tubes containing activated carbon prior to entering vials of deionized water to maintain high ambient humidity. Both of the apparatuses were shielded within large, cardboard enclosures lined with white paper to prevent exposure to external stimuli in the lab (e.g. ambient light entering the room through windows). To create as natural a setting as possible, I mounted a thermostat and 11 W Flexwatt Heat Tape under each olfactometer to maintain constant summer temperatures of approximately 28°C. Additionally, a UVB producing fluorescent bulb (Zoomed: ReptiSun 10.0) was mounted above each olfactometer to simulate natural light without excess heat production.

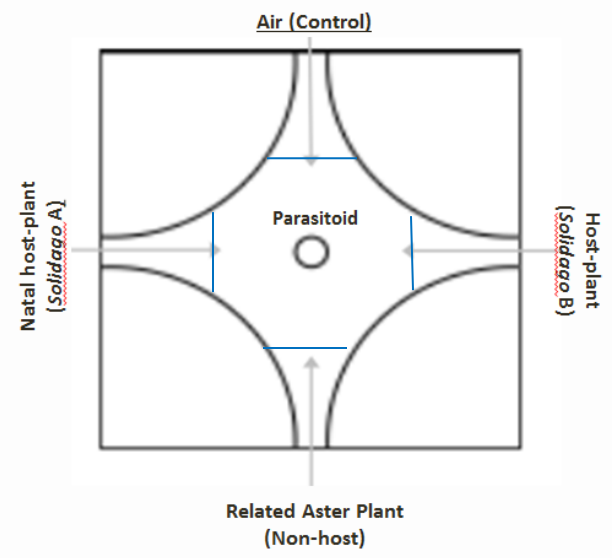

Figure 2. Diagram of the central chamber of the four-choice olfactometer. Arrows represent the uniform flow of air passing through each arm into the central arena where it is drawn out through the central opening. The parasitoids were introduced through the hole in the central opening of the arena. The blue lines are the choice fields, where a choice was scored if a parasitoid entered the odor field past this line.

When selecting wasps for olfactometry experiments, I first had to determine if it was possible to identify both the sex and species of each individual shortly after eclosion from the gall. This proved to be a difficult task, as the parasitoids are minute (1-2.5mm in length) and difficult to identify. Identification to species requires close examination of morphological characters under a dissecting scope. Previous studies have shown that adult female parasitoids exhibit a stronger response to host odors for oviposition (Turlings, 2004; Graziosi & Rieske, 2013) and trials performed by Turlings et al. (2004) using both sexes resulted in males preferentially moving to locations where females had previously visited instead of responding to host cues as desired. Despite these

observations, I still tested the behavioral responses of male parasitoids, because behavioral responses may vary by taxa, and in the event that males have a preference for natal host odors, it might be a factor in isolating host-associated populations to facilitate divergence by isolating gene flow.

To prevent associative learning, each individual parasitoid was used only once per trial in each experimental treatment (Silva et al., 2007). In the event I could not accurately identify the species and sex of living specimens, I conducted trials with 1-4 parasitoids for each experiment that came from the same community breeding container that originated from the same host plant species and collection site. Sex and species identification was determined after olfactometry experiments were complete if it could not be accomplished while parasitoids were living.

Initial trials indicated that the parasitoids were rather sedentary in the olfactory chambers and took substantially longer to make choices than originally anticipated. Thus, I placed individual parasitoids in the 4-choice olfactometer for a trial duration of 90-minutes. Each individual wasp was given 90 minutes to make a choice between air (control), leaves from a natal host plant, leaves from a different related plant species (e.g., *Solidago* or Astereae), and leaves from a non-host in the family Asteraceae (i.e. *Helianthus sp*.). To account for possible directional bias, the olfactometer was rotated 90 degrees between every trial.

The entire olfactometer was disassembled and washed with neutral soap and deionized water between each odor test situation to eliminate faint odors from previous trials that may be detected by parasitoids (Vet et al. 1983). I mounted a high definition video camera (Panasonic HC-V720) above the central chamber to record all behavioral

activity, recording the first choice, time spent in each odor field, and final choice for the parasitoid (see Fig. 2 & Table 2). The first odor field that the parasitoid enters counted as the first choice. As the parasitoid moved, the time spent in each odor field was recorded over the course of a ninety minute trial. If the parasitoid walked into an airflow tube, that odor source was recorded as the final choice and the remaining time left in the assay was credited to that odor field. After the ninety minute trial, if the parasitoid did not enter an airflow tube, the odor field that the parasitoid was located in was recorded as the final choice.

Table 2. Behavioral Olfactometry Experiment

	Trial
Testing Apparatus	Four-choice olfactometer
Odor Treatment #1	Air (Control)
Odor Treatment #2	Leaves from Natal Host Plant (*Solidago* or Asterea)
Odor Treatment #3	Leaves from related *Solidago* or Asterea
Odor Treatment #4	Leaves from non-host Aster (e.g., *Helianthus sp*.)

After conducting olfactometry experiments, both tested and untested parasitoids were placed in a glass vial containing 95% ethanol along with a label containing morpho-species, host plant, gall-morph, and collection information, then stored in a freezer for DNA analysis.

Molecular Methods

DNA was extracted from parasitoids that were collected during this study to amplify and sequence 800 bp of the mitochondrial barcoding gene cytochrome oxidase c subunit I (COI) and nuclear DNA from 850 bp of the internal transcribed spacer gene

(ITS2). Both mitochondrial and nuclear gene sequences were used to create haplotype networks and phylogenetic reconstructions. This combination of two gene markers is necessary because mitochondria are inherited maternally; thus, host-associated genetic structure may be seen in COI, but there remains a possibility that the rest of the genome is more porous to gene flow and thus a nuclear marker is necessary to confirm genetic divergence (Hurst & Jiggins, 2005).

(i) DNA Extraction and Isolation

Due to the minute size of these insects, entire specimens were flash-frozen by placing a tube containing the specimen into liquid nitrogen, and subsequently pulverizing with a pestle prior to chemical treatment. DNA was then extracted using PUREGENE DNA extraction kits (Gentra Systems, Inc., Minneapolis, MN) as described by Stireman et al. (2006).

(ii) mtDNA and nDNA amplification

Once isolated, the mitochondrial DNA was amplified using Polymerase Chain Reaction (PCR) for 450-800 bp of cytochrome oxidase I (COI) along with nuclear DNA from 850 bp of the internal transcribed spacer gene (ITS). DNA was then amplified in 30μl PCR reactions containing 1 μl of genomic DNA, 3 μl (10X) PCR buffer (Invitrogen), 3 μl (10mM) dNTP solution, 4.5 μl (25mM) $MgCl_2$, 1.5 μl of forward and reverse primers (5 pmol μl^{-1}) and dH_2O.

The mitochondrial COI gene was amplified using the forward primer LCO1490 (5'-TAAACTTCTGG ATGTCCAAAAAATCA-3') and reverse primer LepR1 (5'-

GGTCAACAAATCATAAAGATATTGG-3'). PCR was carried out in an Eppendorf Mastercycler gradient using the program DDNUDNA2 under the following PCR conditions: initial denaturing at 94°C for 4mins, 30 cycles of 94°C for 30 sec, 50°C for 1 min, 72°C for 2 min; 30 cycles of of 94°C for 30 sec, 48°C for 1 min, 72°C for 2 min; 30 cycles of 94°C for 30 sec, 45°C for 1 min, 72°C for 2 min and a final extension 72°C period of 4 min.

The non-coding nuclear internal transcriber spacer gene was amplified in the ITS2 region between the 5.8s and 28s genes using the forward primer CAS5p8sFc (5'-TGAACATCGACATTTYGAACGCACAT-3') and reverse primer CAS28sB1d (5'-TTCTTTTCCTTCCSCTTAYTRATATGCTTA-3'). PCR was performed in an Eppendorf Mastercycler gradient using the program ITS-HYM-1 under the following PCR conditions: initial denaturing at 94°C for 4mins, 35 cycles of 95°C for 20 sec, 62°C for 40 sec, 72°C for 20 sec; and a final extension 72°C period of 2 min post-cycles (Ya-Jei et al., 2003).

(iii) Agarose Gel Electrophoresis

After amplification of the desired sequences, products were verified using agarose gel electrophoresis. A 1.5% agarose gel was prepared and treated with ethidium bromide for ultraviolet detection of DNA product. Each well contained a 4 µl mixture of 2 µl of PCR product and 2 µl of 6x blue gel loading dye. Samples were run for 50 minutes at 80V, and were then exposed to ultraviolet light to reveal bands of separated DNA. Photographs of the gels were taken and samples that revealed a positive product (bands) were selected for sequencing.

(iv) Sequencing and Editing

Primer aliquots and 27μl of PCR product from samples that were verified to yield amplified DNA were plated in a 96-well plate and sent to the University of Arizona Genetics Core for sequencing using Applied Biosystems 3730 DNA Analyzers. Manual editing of sequences was performed using the program Codon Code Aligner (CodonCode Corporation). Alignment was performed with the software MEGA 6.0 using default settings of the alignment algorithm option, MUSCLE (Tamura et al., 2011). Some of the taxa had heterozygous indels for the ITS2 locus and these were processed using the "Process Heterozygous Indels" in Codon Code Aligner. Haplotype phase was also inferred using PHASE v2.1 in DnaSP and the phased heterozygous alleles were treated as separate haplotypes in analyses (Librado & Rozas, 2009; Stephens & Donnelly, 2003).

(*v*) *Haplotype Networks and Phylogenetic Analysis*

Haplotype networks were constructed using the NETWORK program package (distributed by www.fluxus-engineering.com) from sequence data from both COI and ITS2 in order to estimate genealogical associations in relation to host plant species (as described by McLeish et al., 2012).

COI and ITS2 sequence data was used to develop a phylogenetic reconstruction for each parasitoid taxon. The software MEGA 6.0 was used to build maximum likelihood (ML) phylogenetic trees using the bootstrap method under 1000 replications (Tamura et al., 2011). I calculated the best-fit Maximum Likelihood nucleotide model for each gene in each parasitoid data set in MEGA to find that the T92+G+1 substitution

model returned the lowest Akaike Information Criterion (AIC) for all samples tested. A model with the smallest AIC is generally favored, because it may be interpreted as the amount of information lost when a particular model approximates the real process of a nucleotide substitution (Posada & Buckley, 2004). For the treatment of missing data, all sites were treated with partial deletion (Hall, 2013). Representative outgroups were not available on public databases for the ITS gene for any of the parasitoids. Outgroups for COI were included in the construction of the ML-trees and are described in figure legends but only the species-level subtree is shown to better illustrate underlying structure.

(*vi*) *Statistical Analyses*

Olfactometry Data Analysis

I recorded the number of observations of parasitoids making a choice between either natal host or other choice for each species and sex in the behavioral study. All parasitoid species were also lumped together for a total number of responses by males and females of the parasitoid community. I calculated a one-tailed binomial probability for each sex in each parasitoid species and for all species grouped where a 0.25 probability was assigned to the success of a single trial for natal host plant choices in the four-choice olfactometer.

Molecular Data Analysis

To estimate the proportion of genetic variation explained by host and site (geography), and that explained by within-population variability, I computed a two-factor

analysis of molecular variance (AMOVA) in Arlequin 3.0 by nesting host plant within site and vice versa (Excoffier et al., 2005; Stireman et al., 2005). The fixation index (F_{ST}) and P-values (0.05 significance level under 1023 permutations) were estimated in this AMOVA**,** where an F_{ST} of 0 would indicate complete panmixia of the populations freely interbreeding, while a value approaching 1 indicates complete isolation of populations. Note that interpretation of Fst is limited when sample sizes are small or vary between populations.

Genetic Distance Matrices

Mean pairwise genetic distances between parasitoids grouped by natal host plant were calculated in MEGA 6.0 with a Kimura two-parameter model (Tamura et al., 2011; Egan et al., 2013). The mean distance between groups is the arithmetic mean of all pairwise distances between the two groups from different natal hosts. COI and ITS2 were concatenated in parasitoids when computing pairwise distances between host plant groups. Distances for COI in *Asteromyia carbonifera* host-associated races and ITS distances in host plants (*Solidago*, *Euthamia*, and *Symphyotrichum*) were also generated in pairwise distance matrices (*Asteromyia* distances retrieved from Stireman et al., 2012; plant distances retrieved from Laureto & Barkman, 2011). These distance matrices provide a visual comparison of genetic distance between host-associated lineages in parasitoids, host-associated lineages in midges, and host plants.

Mantel Tests

In order to determine how well correlated the genetic matrices were with one another, I performed mantel tests by using the 'Mantel Test' application in the program, XLSTAT (Millar, 2001). Mantel tests were run with 10,000 randomizations and one-tailed hypothesis testing. If parasitoids are diverging with the midges, I would expect to see a stronger correlation (r(AB)) between the midge distances and parasitoid distances, while divergence with host plant might reveal a correlation between plant distances and parasitoid distances.

RESULTS

Behavioral Responses to Host Plant Odors

A total of 60 trials were conducted in the 4-choice olfactometer, with 48 trials resulting in either a natal or non-natal host choice and 12 trials resulting in no choice (Table 4). Due to the small size and similar appearance of the wasps to the unaided eye, it was rarely possible to determine the species of parasitoid being tested in the olfactometer while they were living. This created a challenge when attempting to achieve a sample size to effectively evaluate odor responses, because there are 4 possible choice to be made in the olfactometer, up to 7 possible natal host plants to consider, and 6 species of parasitoids. In addition, parasitoid life expectancy is limited (~3-6 days), which made testing challenging during periods when there were higher numbers of eclosions. For example, during the first week of August 2014, nearly half of the galls collected up to that date had insect emergence within a short window of time, so a large proportion of wasps perished before having a chance to be tested. Despite these challenges, I found strong evidence of biased orientation towards natal hosts from a one-tailed binomial probability test when all of the parasitoid species were lumped for analysis (Table 4). In addition, female *Aprostocetus tesserus*, *Aprostocetus sp. "T1"*, and *Platygaster solidaginis* all showed significant preferences for natal host plant genera, but sample sizes at the species-level were low, particularly for males (Tables 5-7).

Table 3. Reference list of shortened 'code' names for all parasitoid species, collection sites, plant species, and gall morphotypes. Code names are used in a variety of the result outputs to consolidate space.

Code	Parasitoid Species
T1	*Aprostocetus sp. "T1"*
tess	*Aprostocetus tesserus*
bary	*Baryscapus fumipennis*
clos	*Closterocerus solidaginis*
tory	*Torymus capitis*
platy	*Platygaster solidaginis*
Code	**Collection Site**
Ade	Adelphi, OH
Crn	Crane Hollow Reserve, Athens, OH
Hmp	Huffman MetroPark, Dayton, OH
Kis	Kiser Lake, St Paris, OH
Oak	Oakes Quarry Park, Fairborn, OH
Fen	Siebenthaler Fen, Beavercreek, OH
Fac	Creekside Reserve, Beavercreek, OH
Jbw	Beavercreek (Private Property), OH
Gsp	Great Seal State Park, Chillicothe, OH
Stt	Yellow Springs (Private Property), OH
Kwra	Kentucky Wildlife Refuge Area, Richmond, KY
Ntt	New Tazewell, TN
Dhl	Dale Hollow Lake, Celina, TN
Code	**Plant Species**
alt	*Solidago altissima*
gig	*Solidago gigantea*
jun	*Solidago juncea*
nem	*Soldidago nemoralis*
pat	*Solidago patula*
Eut	*Euthamia graminifolia*
Sym	*Symphyotrichum lanceolatum*
Code	**Gall Morph**
cre	Crescent
cus	Cushion
fla	Flat
gra	Graphite
irr	Irregular
pim	Pimple

Table 4. Contingency table of observed olfactory responses by parasitoids. All species were lumped due to limited sampling to better understand the general responses of the parasitoid community as a whole. Only individuals that made a choice were considered (N=48). Significant (P<0.05) binomial probabilities are bolded and given an asterisk.

All Parasitoid Species Lumped				
	Natal	Other	Total	Binomial Probability P(X = x)
Female	23	13	36	**<0.000001***
Male	4	8	12	0.193577
Total	27	21	48	**0.000003***

Table 5. Contingency table of observed olfactory responses by female *Aprostocetus sp. "T1".* Only individuals that made a choice were considered (N=5). Significant (P<0.05) binomial probabilities are bolded and given an asterisk.

***A. sp. 'T1'* (Female) - Choices and Host Plant Origin**			
Host Plant	Natal Choice	Other Choice	Binomial Probability (PX=x)
alt	2	0	-
gig	0	1	-
Eut	1	0	-
Sym	1	0	-
Total	4	1	**0.014648***

Table 6. Contingency table of observed olfactory responses by female *Aprostocetus tesserus.* Only individuals that made a choice were considered (N=13). Significant (P<0.05) binomial probabilities are bolded and given an asterisk.

***A. tesserus* (Female) - Choices and Host Plant Origin**			
Host Plant	Natal Choice	Other Choice	Binomial Probability (PX=x)
alt	0	2	-
gig	0	2	-
jun	0	1	-
Eut	3	0	-
Sym	4	1	-
Total	7	6	**0.01864***

Table 7. Contingency table of observed olfactory responses by female *Platygaster solidaginis.* Only individuals that made a choice were considered (N=4). Significant (P<0.05) binomial probabilities are bolded and given an asterisk

***Platygaster solidaginis* (Female) - Choices and Host Plant Origin**			
Host Plant	Natal Choice	Other Choice	Binomial Probability (PX=x)
jun	2	0	-
Eut	1	0	-
Sym	1	0	-
Total	4	0	**0.003906***

Host-associated Genetic Structure

Some parasitoid species displayed quite strong genetic structure according to host plant but this was not always the case. I will present evidence of host-associated structure

in those with the most compelling evidence first, followed by those with decreasing evidence of host-associated genetic structure.

Table 8. AMOVA for parasitoid community with either host plant (of host insect) nested within geography or vice versa. Analyses considered either all host plants (All) or only *Solidago* hosts (Solidago) to better examine variation explained by host plant within-genus in the event different host plant genera are driving the majority of the variation. Significant values ($P<0.05$) are bolded and given an asterisk.

		COI				ITS			
	Species	Host	Site	Within Pops.	F_{st}	Host	Site	Within Pops.	F_{st}
Site nested within host	Bary (Solidago)	**67.24***	**22.66***	10.11	0.89	**59.97***	18.99	21.04	0.68
	T1 (All)	**91.91***	-21.72	**29.81***	0.7	-16.5	103.52	12.97	0.87
	T1 (Solidago)	76.33	-33.97	57.64		77.57	-35.41	57.84	
	Tess (All)	5.29	27.06	67.65	0.32	28.87	-31.21	102.34	0.23
	Tess (Solidago)	4.05	46.41	49.54		21.37	-10.52	89.15	
	Platy (All)	**74.70***	1.34	**10.11***	0.84	3.95	23.21	72.48	0.25
	Platy(Solidago)	-16.08	31.66	84.42		-1.75	**27.23***	74.48	
	Tory (Solidago)	-5.37	**34.41***	70.96	0.29	-7.26	9.05	98.2	0.17
	Clos (All)	-	-	-	-	34.26	-17.43	83.16	0.16
Host nested within site	Bary (Solidago)	**61.45***	29.24	9.31	0.68	47.74	16.36	68.62	0.51
	T1 (All)	59.72	30.66	19.62	0.63	**53.20***	**36.33***	10.47	0.69
	T1 (Solidago)	55.76	35.34	-19.89		48.31	55.90	-4.21	
	Tess (All)	9.03	19.85	71.12	0.26	10.71	24.76	64.53	0.35
	Tess (Solidago)	7.55	48.77	44.68		19.67	16.98	63.35	
	Platy (All)	53.88	10.34	19.56	0.31	13.3	3.94	82.75	0.17
	Platy (Solidago)	-21.72	31.66	68.98		-11.25	33.12	78.13	
	Tory (Solidago)	-5.32	**38.43***	**66.89***	0.22	-8.18	13.01	86.99	0.23
	Clos (All)	-	-	-	-	6.48	4.19	89.33	0.22

a) Baryscapus fumipennis

A total of 33 and 32 sequences were successfully amplified in *Baryscapus fumipennis* for COI and ITS2, respectively. Distinct host-plant associated clusters formed in the COI tree within the *Solidago* genus, with a separation between the closely-related *S. altissima* and *S. gigantea* (77% bootstrap support) and other *Solidago* plant hosts (82% bootstrap support; Figure 3). Within the other *Solidago* hosts, strong support was found in separation of host-plant associated clusters for *S. juncea, S. patula,* and *S. nemoralis* (>75%). The COI median-joining network also provides strong evidence of underlying host-associated structure in the haplotypes (Figure 3). The AMOVA revealed a very high F_{st} in COI (0.89) when site was nested in host, supporting isolation of populations and significant contributions to genetic variation by both host plant (67.24%) and site (22.66%) for COI.

The ML tree for the ITS2 region depicts some host-associated structure, with general clustering by species. Bootstrap support was modest (<75%) at the majority of branches (Figure 4). The haplotype network for ITS also depicts a general separation by host plant (Figure 6). The AMOVA shows a fairly high F_{st} (0.68) for ITS with significant contributions to genetic variation by host plant (59.97%; Table 8).

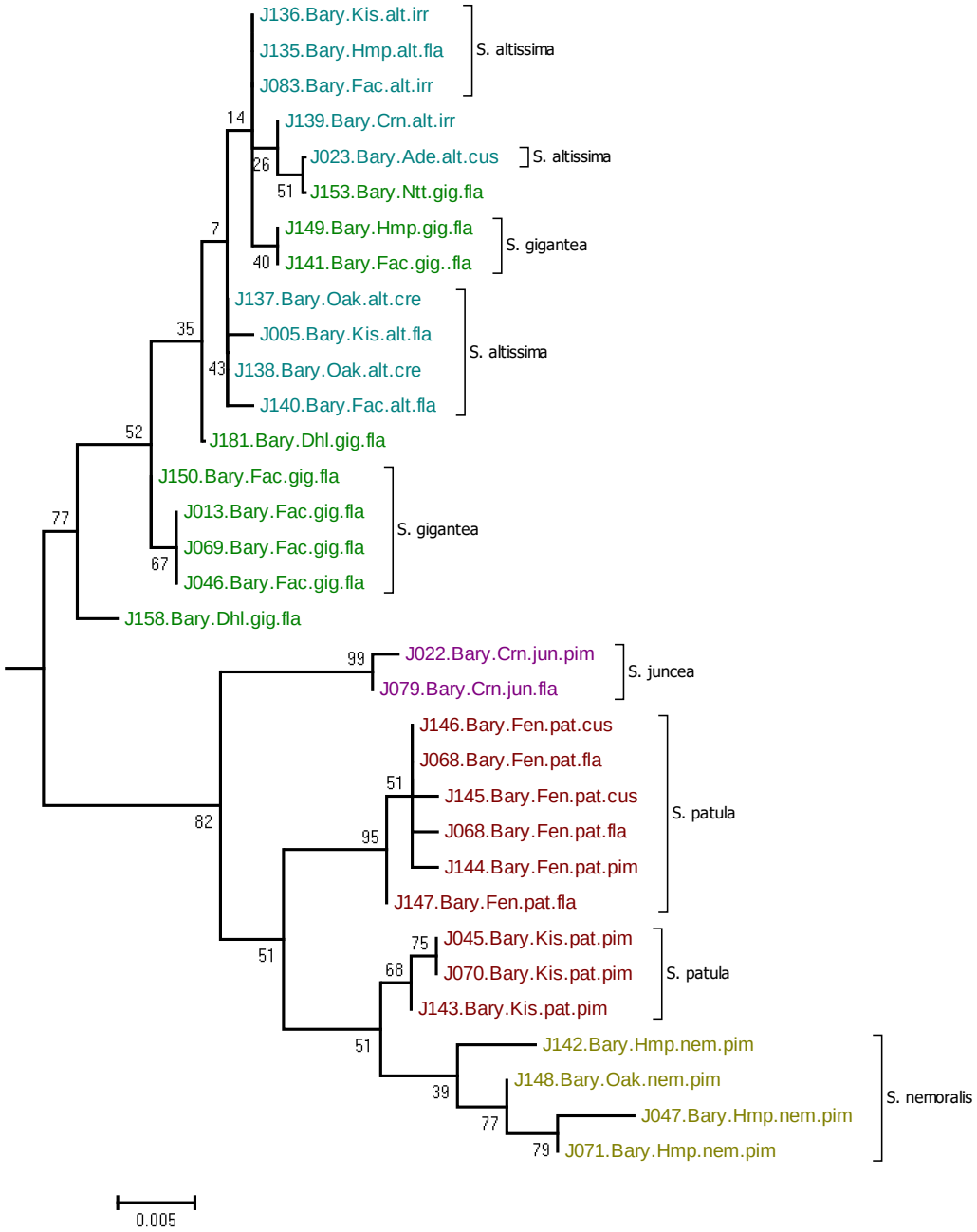

Figure 3. Maximum likelihood subtree of *Baryscapus fumipennis* for the COI gene using the T92+G+I model and 1000 bootstraps. The bootstrap support values are located to the left of each node. The branches are colored according to host-plant as follows: *Solidago altissima*: teal; *Solidago gigantea*: green; *Solidago patula*: red; *Solidago juncea*: purple; *Solidago nemoralis*: olive/yellow. The outgroup, *Aprostocetus* sp."T1", was excluded

from this subtree to better illustrate within-species structure. Refer to Table 3 for sample codes.

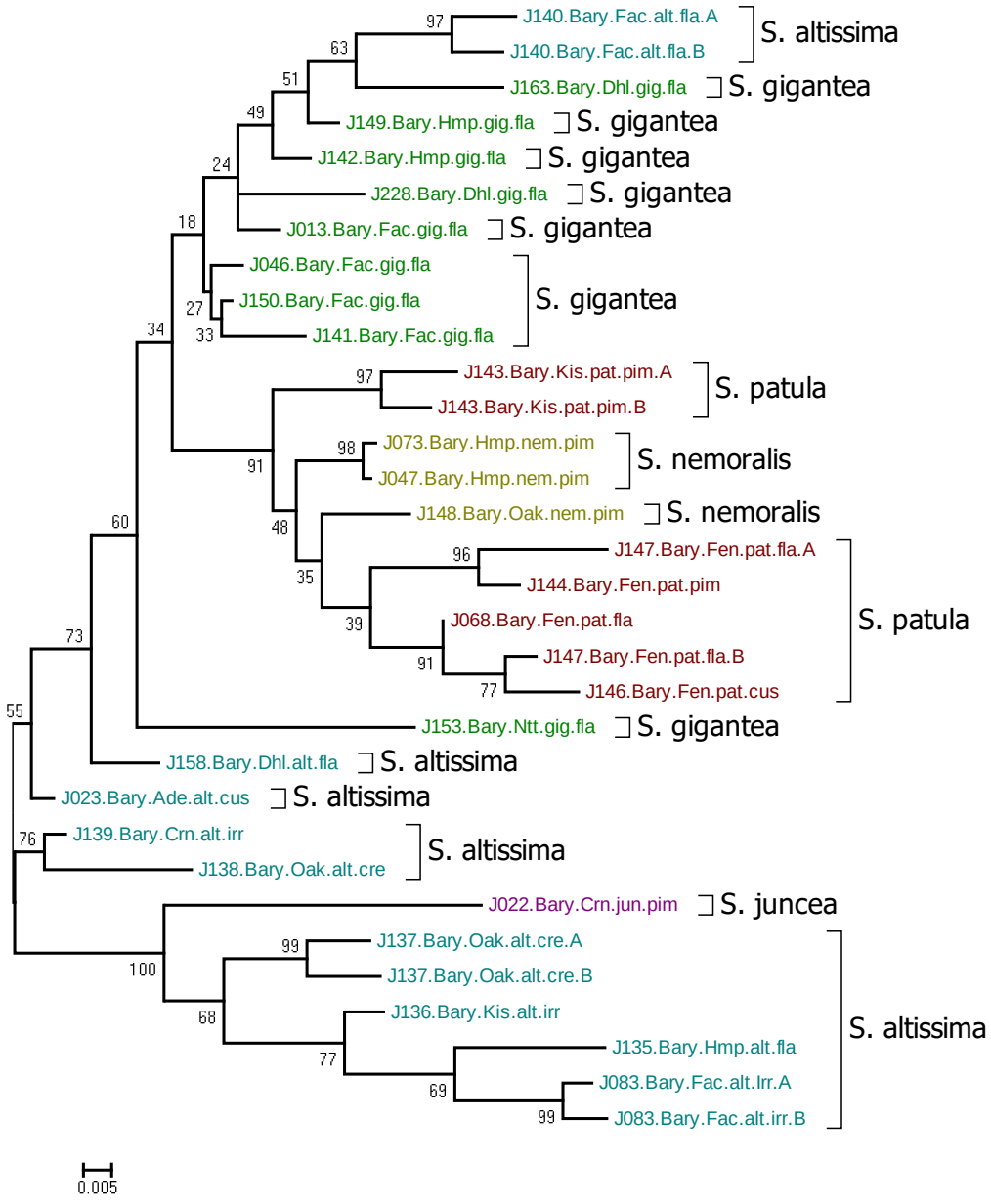

Figure 4. Maximum likelihood subtree of *Baryscapus fumipennis* for the ITS gene with 1000 bootstraps. All taxon code labels and branch colors are the same as Figure 3.

Heterozygotes were treated as separate haplotypes and are depicted at the end of the taxon code with an “A” or “B”.

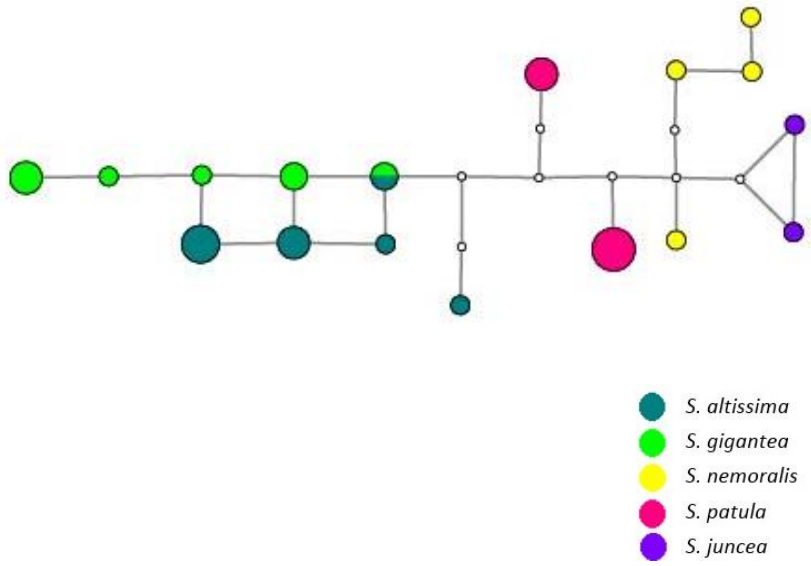

Figure 5. Haplotype network constructed by median joining of *Baryscapus fumipennis* COI sequences.

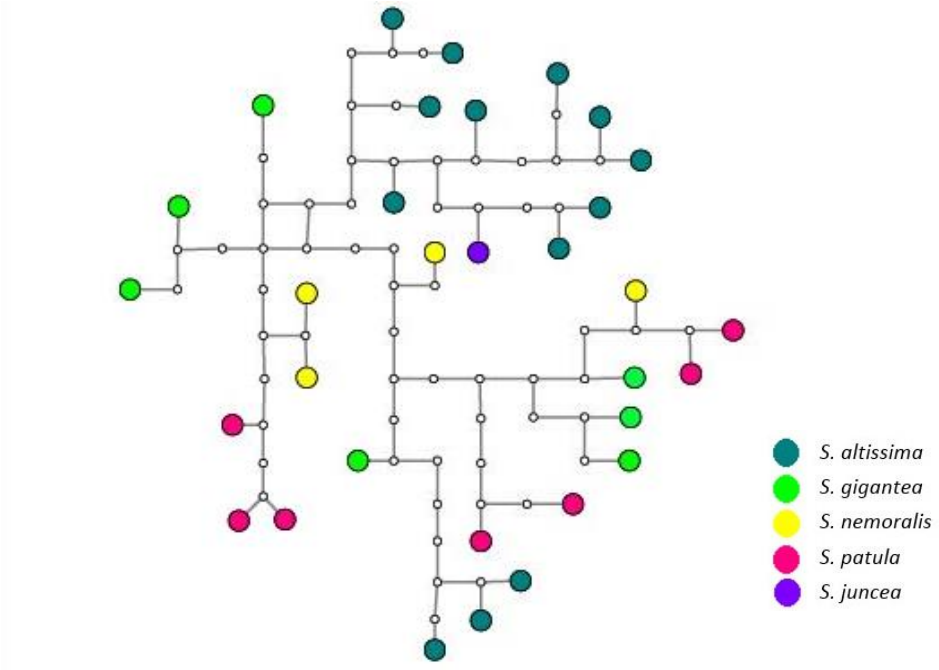

Figure 6. Haplotype network constructed by median joining of *Baryscapus fumipennis* ITS sequences.

b) Platygaster solidaginis

A total of 21 and 32 sequences were successfully amplified in *Platygaster solidaginis* for COI and ITS2, respectively. Phylogenetic reconstructions were conducted in the same manner as *Baryscapus fumipennis*. Distinct host-plant associated clusters formed in the COI ML tree with well-supported separation by host-plant genera (Figure 7). A distinct *Solidago* clade arose with 95% bootstrap support along with a grouping of *Symphyotrichum* and *Euthamia* (95% bootstrap support). *Euthamia* and *Symphyotrichum* cluster at the genus level within that clade despite relatively low support at the branches.

The median-joining network for COI illustrates the separation by host-plant genera very well among haplotypes (Figure 8). AMOVA returned a high F_{st} (0.84) and resulted in significant contributions to genetic variation in COI by host plant when all plant species were considered (74.70%) but not when only *Solidago* hosts were examined (Table 8). This suggests that variation is driven by host plants at the genus-level.

The ML tree and haplotype network for the ITS2 region depicts minor host-associated structure with some weakly supported clustering by host plant genera (Figure 8, Figure 10). A significant value by geography in ITS was returned where site explained 27.23% of the variation when site was nested in host but there were otherwise no significant values in either of the AMOVA analyses.

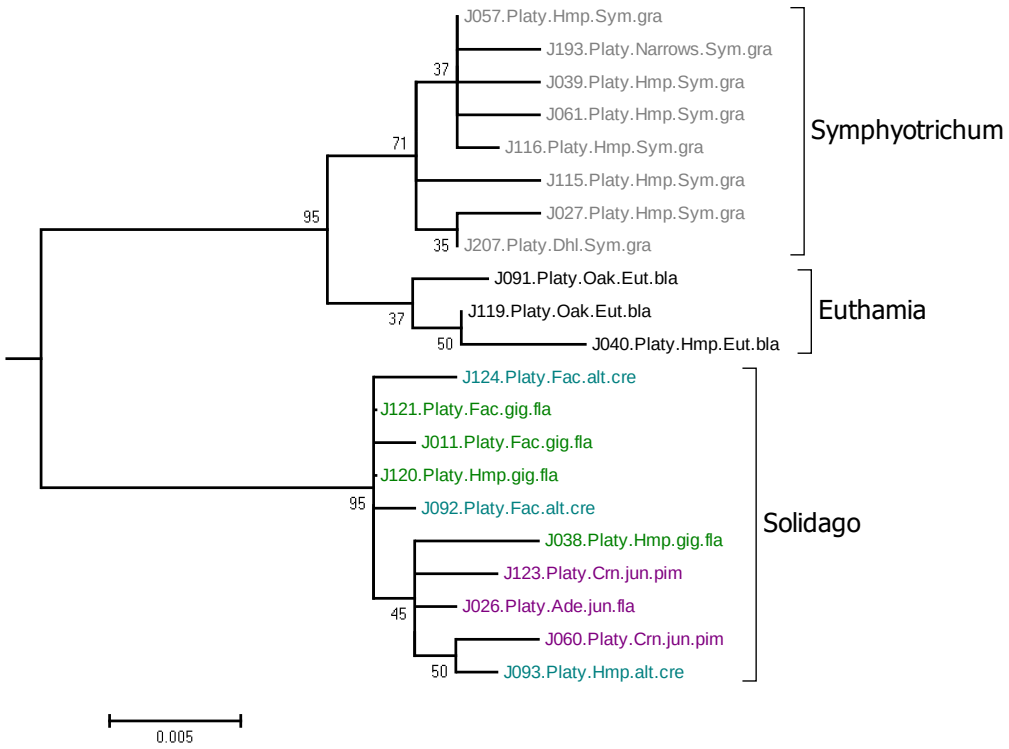

Figure 7. Maximum likelihood subtree of *Platygaster solidaginis* for the COI gene with 1000 bootstraps. All taxon code labels and branch colors are the same as Figure 3.

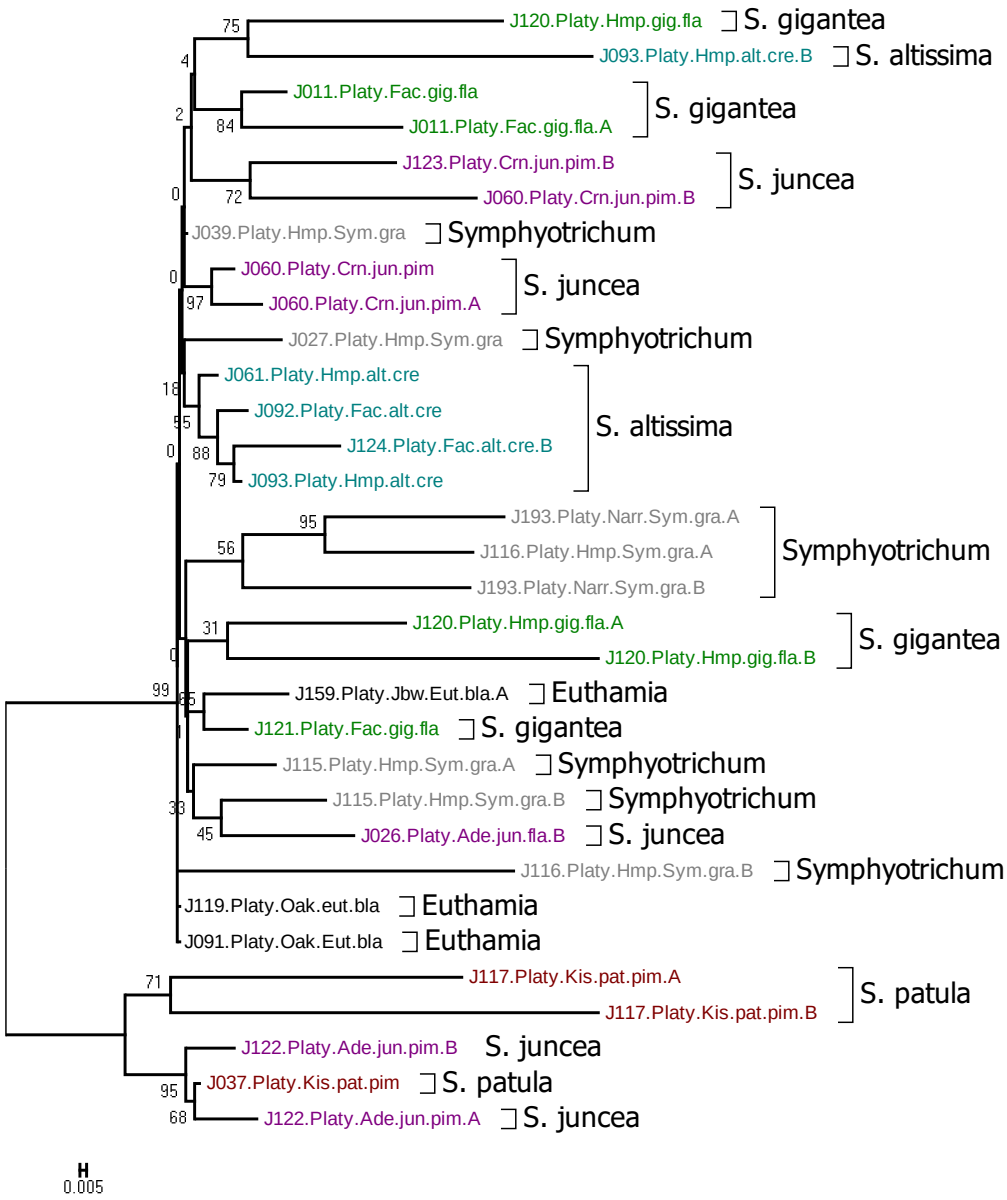

Figure 8. Maximum likelihood subtree of *Platygaster solidaginis* for the ITS gene with 1000 bootstraps. All taxon code labels and branch colors are the same as Figure 3. Heterozygotes were treated as separate haplotypes and are depicted at the end of the taxon code with an "A" or "B".

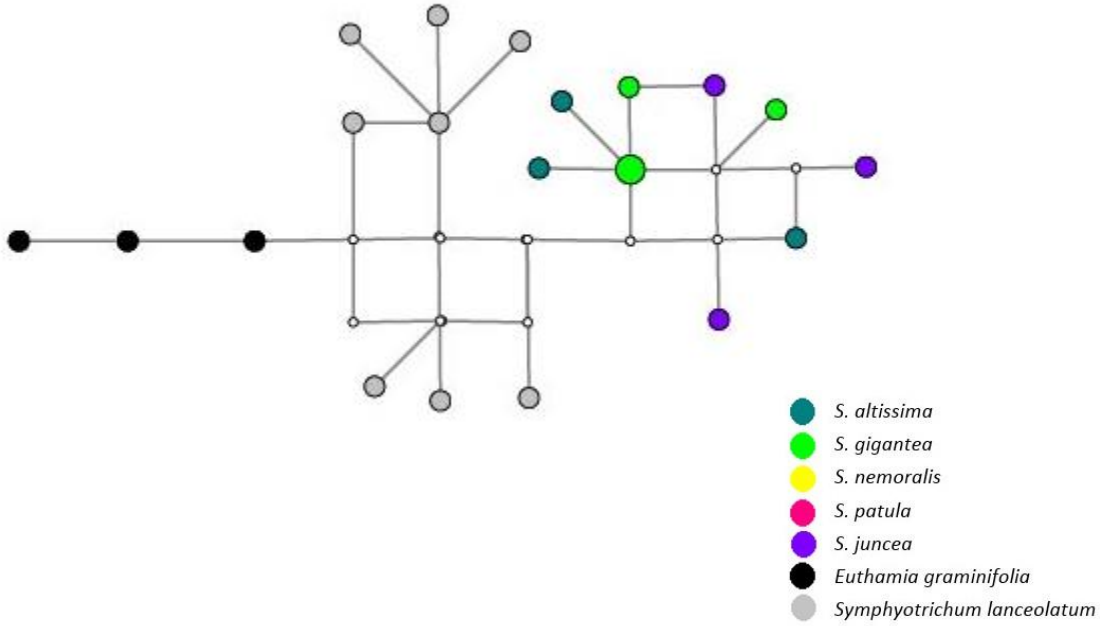

Figure 9: Haplotype network constructed by median joining of *Platygaster solidaginis* COI sequences.

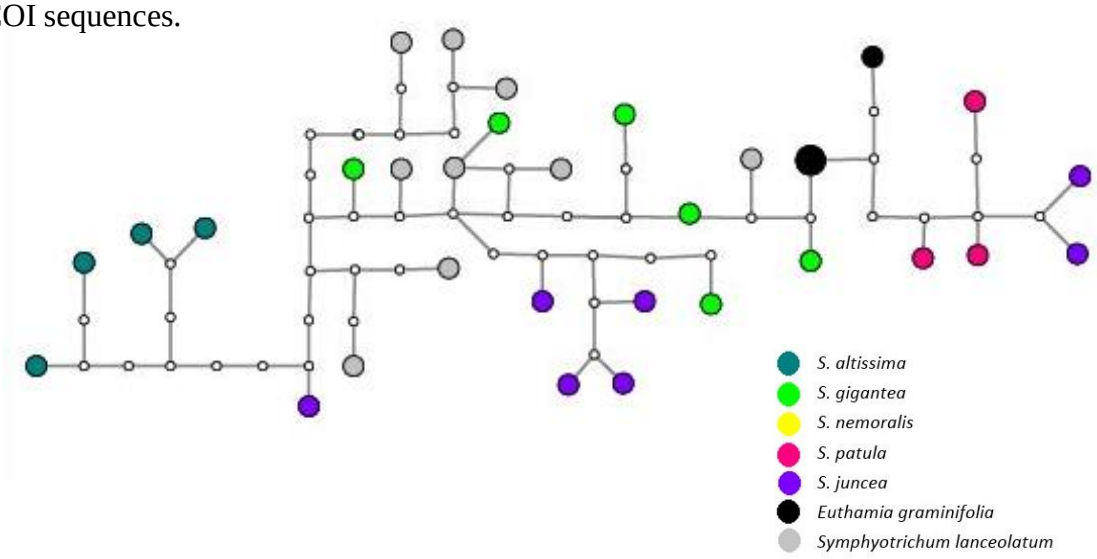

Figure 10: Haplotype network constructed by median joining of *Platygaster solidaginis* ITS sequences.

c) *Aprostocetus sp. 'T1'*

Aprostocetus sp. 'T1' was one of the rarer species collected from *Asteromyia*'s parasitoid community. Only 14 COI sequences and 17 ITS sequences (including 3 heterozygotes) were successfully amplified for *A. sp. "T1".* Distinct host-plant associated groups were recovered at the species-level in the COI ML tree but support is generally quite low (Figure 11). There appears to be deeper divergence between host-plant genera, but the tree and haplotype network both suggest genetic structure associated with host plant species (Figure 13). The AMOVA resulted in a significant 91.91% contribution to genetic variation by host plant in COI when all plant genera were considered in the analysis (Figure 8). However, I failed to find significant evidence when considering *Solidago* hosts alone. The same analysis yielded a significant contribution by within-species variation as well (29.81%).

The ML tree for the ITS2 region also suggests strong host-associated structure at both the genus and species level (Figure 12). The only exception occurs at a polytomy where a sample from *S. juncea* is nested within an *S. patula* clade. Bootstrap support is relatively low, and a larger number of samples from each representative host will be needed to make robust inferences about these data. Haplotype networks for both genes reflected the structure seen in the ML trees well (Figures 13 & 14). When all plants were considered, a relatively high F_{st} was found for ITS when site was nested in host (0.87) and when host was nested in site (0.69). A significant contribution to genetic variation by host (53.20%) and site (36.33) were returned in the AMOVA for ITS when host was nested in site, but interpretation is limited due to small sample size. Additional

representatives of this species from each host at different sites will be needed to better evaluate their genetic structure and its causes.

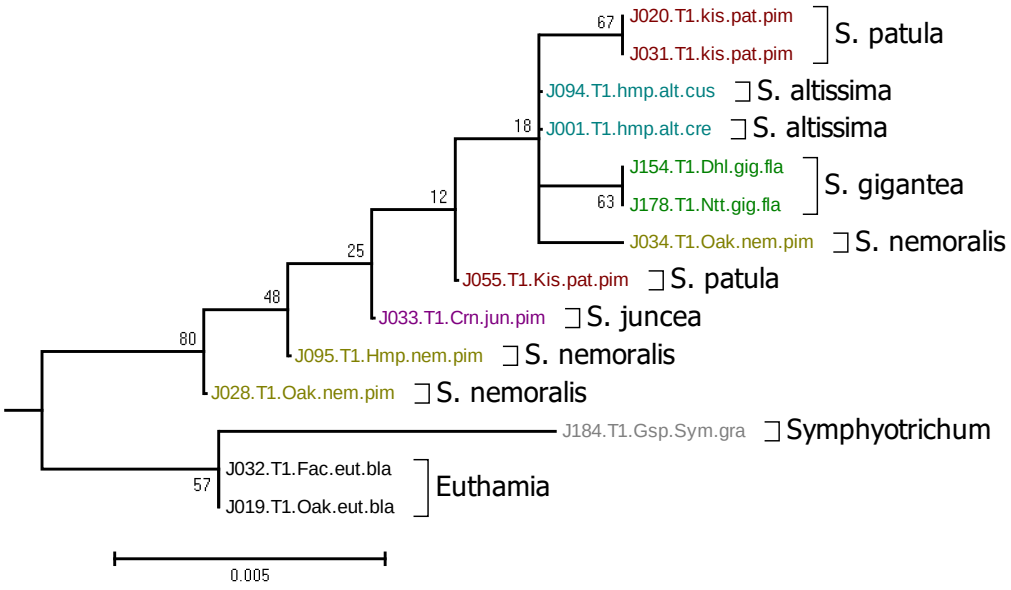

Figure 11. Maximum likelihood subtree of *Aprostocetus sp. 'T1'* for the COI gene using 1000 bootstraps. All taxon code labels and branch colors are the same as Figure 3.

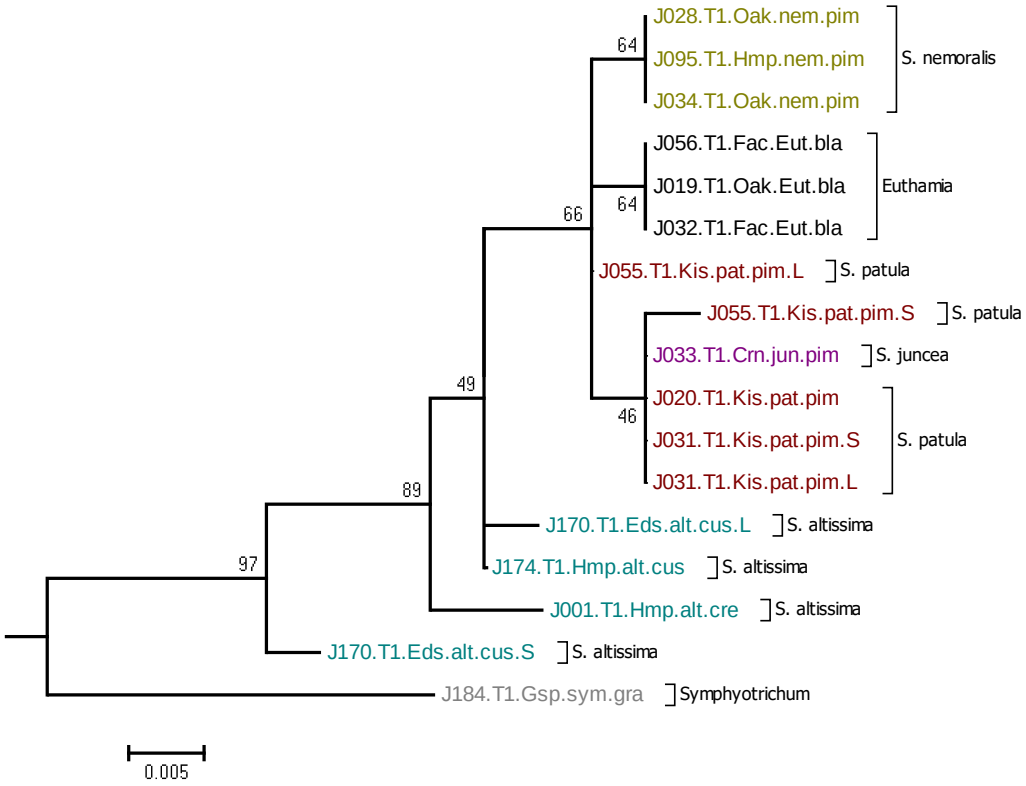

Figure 12. Maximum likelihood subtree of *Aprostocetus sp. 'T1'* for the ITS gene using 1000 bootstraps. All taxon code labels and branch colors are the same as Figure 3.

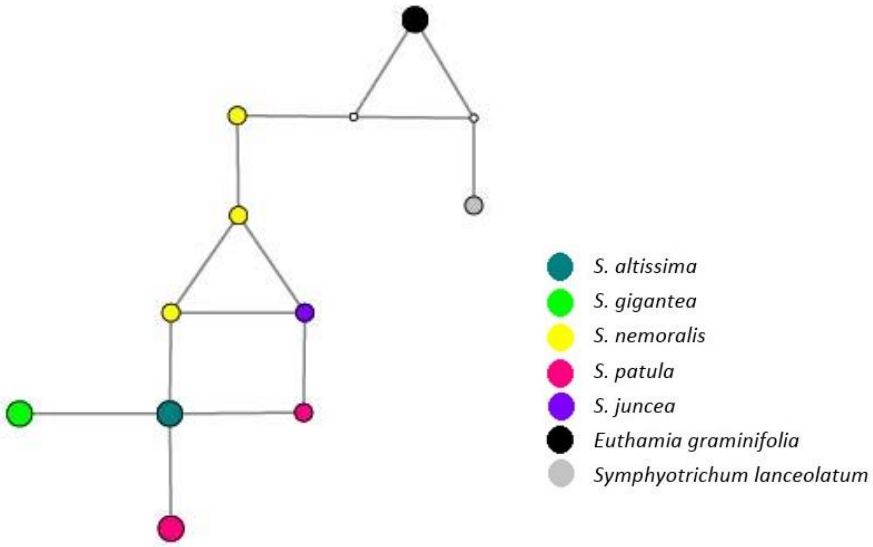

Figure 13. Haplotype network constructed by median joining of *Aprostocetus sp. 'T1'* COI sequences.

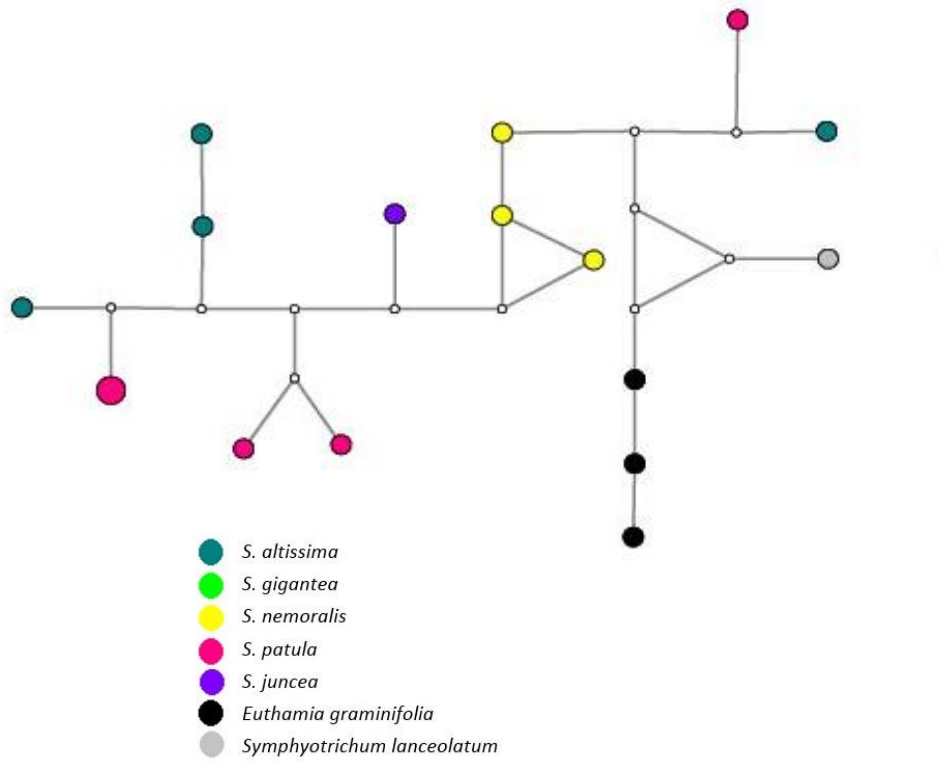

Figure 14. Haplotype network constructed by median joining of *Aprostocetus sp. 'T1'* ITS sequences.

d) Aprostocetus tesserus

Aprostocetus tesserus was the most abundantly collected parasitoid. 31 sequences for both COI and ITS2 were amplified. In both the ML tree and haplotype network for COI, very little underlying host-associated structure was detected (Figure 15 & 17). In the haplotype network, some host plant clustering occurs in *Euthamia* and *Symphyotrichum*, yet haplotypes from these hosts are also found mixed with other populations (Figure 17). Results of AMOVA analyses supported observations in the trees

and haplotype networks (Table 8). The F_{st} value for COI was 0.32 when site was nested in host and no significant contribution was found by either host or site in either AMOVA analysis.

The ML tree for the ITS2 region revealed a *Symphyotrichum* clade with 51% bootstrap support (Figure 15). Otherwise, there is complete admixture among the samples with no apparent structure in *Solidago* or *Euthamia* hosts. The haplotype network for ITS shows general mixing with no apparent clustering (Figure 18). AMOVA analyses supported observations in the trees and haplotype networks (Table 8). The value for F_{st} was 0.32 for ITS2 when site was nested in host. A large proportion of the variation was attributed to within-group when all host plants were considered in ITS, suggesting no host-associated structure.

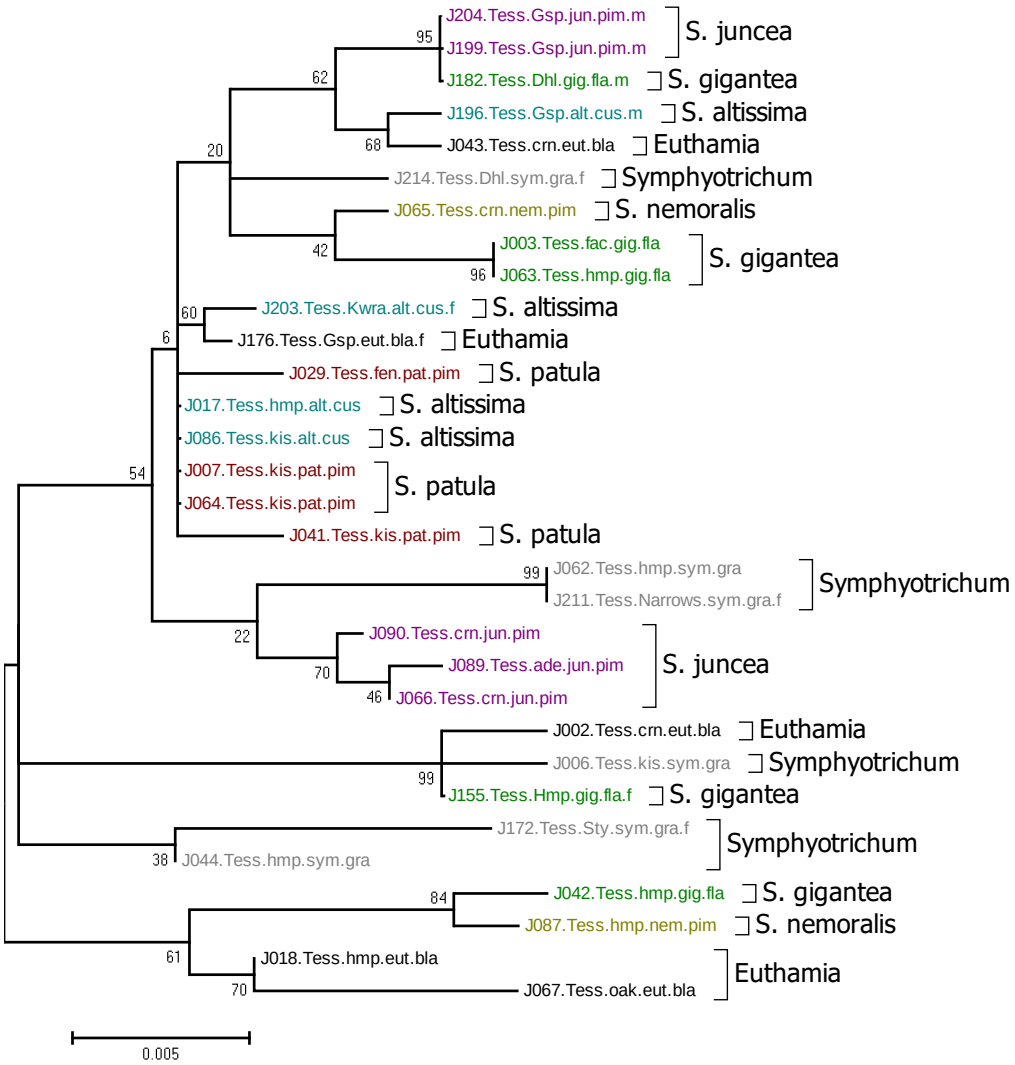

Figure 15. Maximum likelihood subtree of *Aprostocetus tesserus* for the COI gene using 1000 bootstraps. All taxon code labels and branch colors are the same as Figure 3.

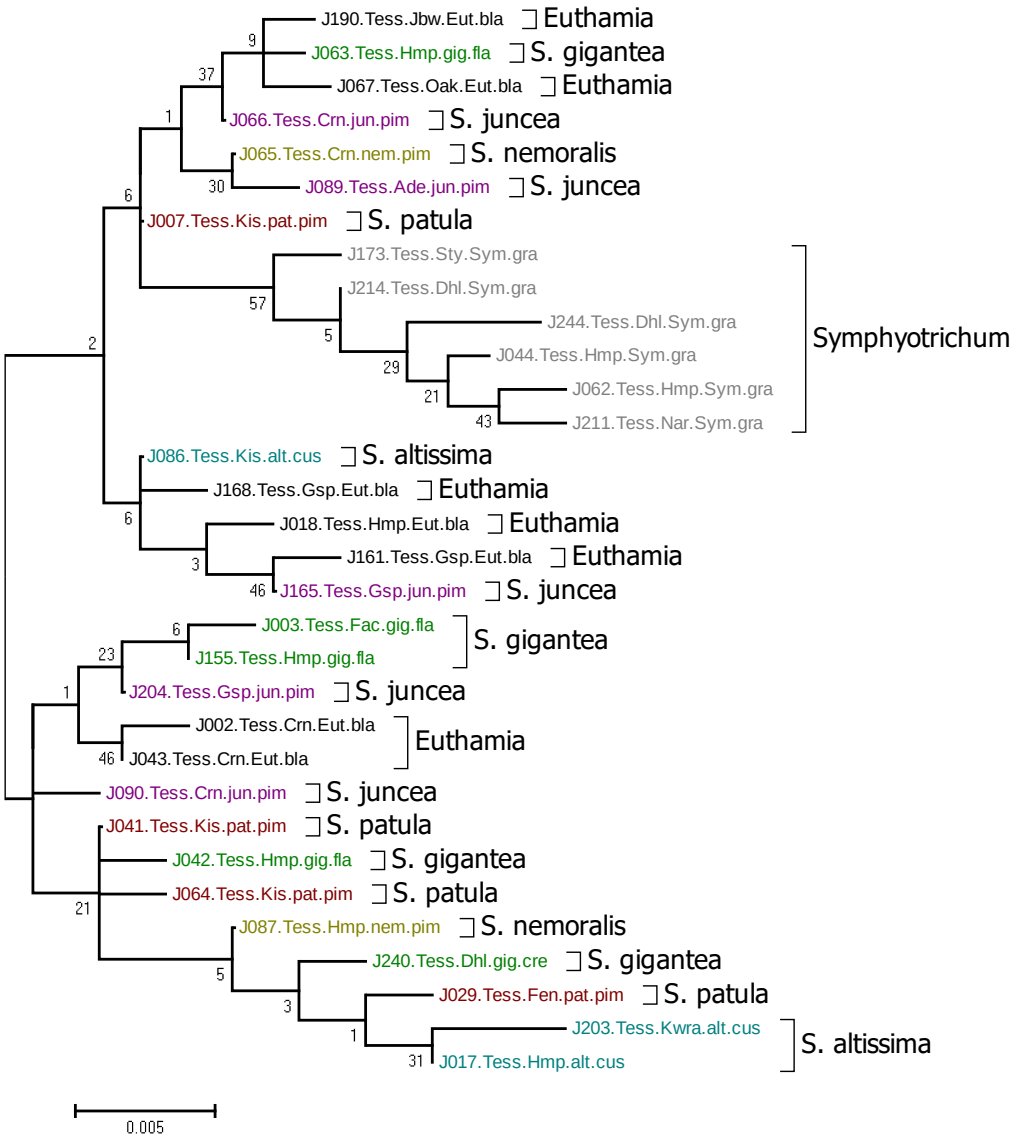

Figure 16. Maximum likelihood subtree of *Aprostocetus tesserus* for the ITS gene using 1000 bootstraps. All taxon code labels and branch colors are the same as Figure 3.

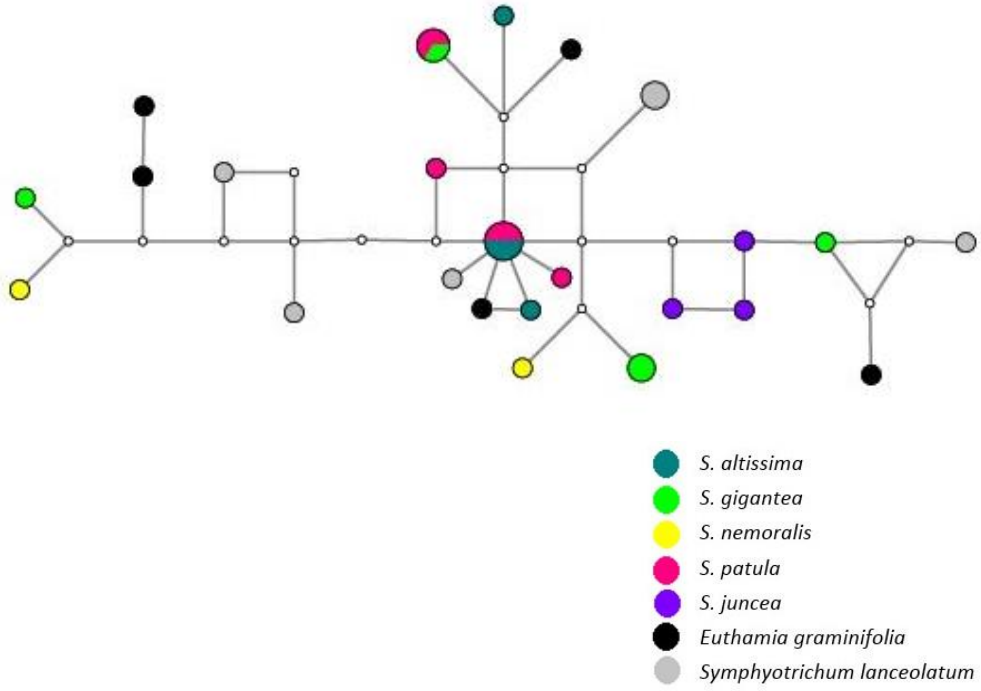

Figure 17. Haplotype network constructed by median joining of *Aprostocetus tesserus* COI sequences.

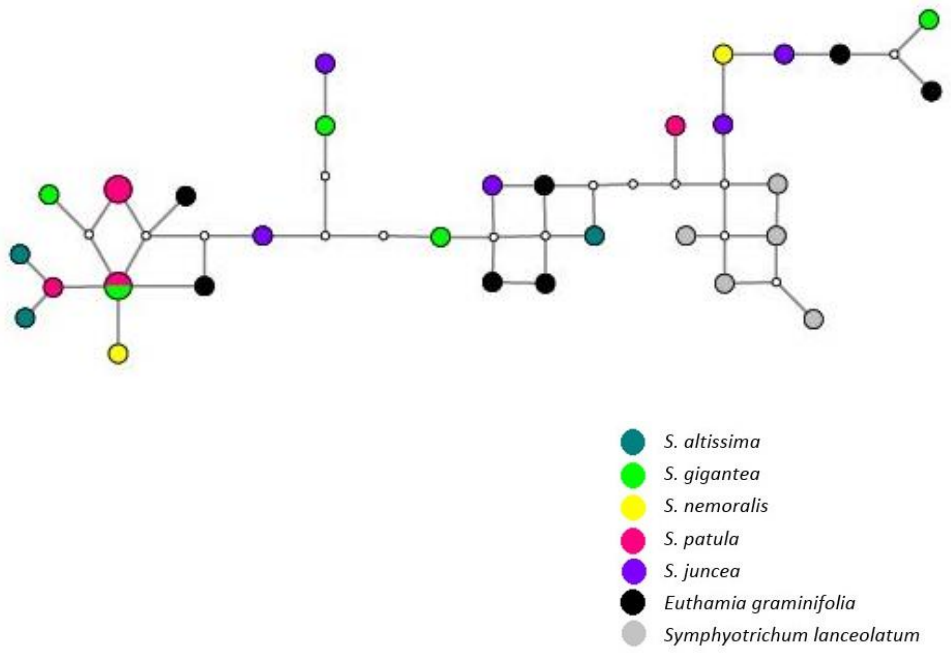

Figure 18. Haplotype network constructed by median joining of *Aprostocetus tesserus* ITS sequences.

e) Torymus capitis

A total of 21 sequences for COI and 31 sequences (2 heterozygotes) from ITS were amplified in *Torymus capitis*. This species was only collected from galls that originated from two closely-related host plants, *Solidago altissima* and *Solidago gigantea*. No apparent structure is found by host in either of the ML trees nor the median-joining networks for COI and ITS (Figures 19-22). There appears to be some clustering as a result of locality, and this is supported by the AMOVA analysis in COI with a significant 34.41% contribution to variation by site (Table 8). The F_{st} results suggest that there is some mating between populations where the F_{st} for COI was 0.29 and 0.17 for ITS2 when site was nested in host.

Figure 19. Maximum likelihood subtree of *Torymus capitis* for the COI gene using 1000 bootstraps. All taxon code labels and branch colors are the same as Figure 3. This species was only collected from two closely related host plants, *S. altissima* (teal) and *S. gigantea* (green).

Figure 20. Maximum likelihood subtree of *Torymus capitis* for the ITS gene using 1000 bootstraps. All taxon code labels and branch colors are the same as Figure 1. This species was only collected from two closely related host plants, *S. altissima* (teal) and *S. gigantea* (green).

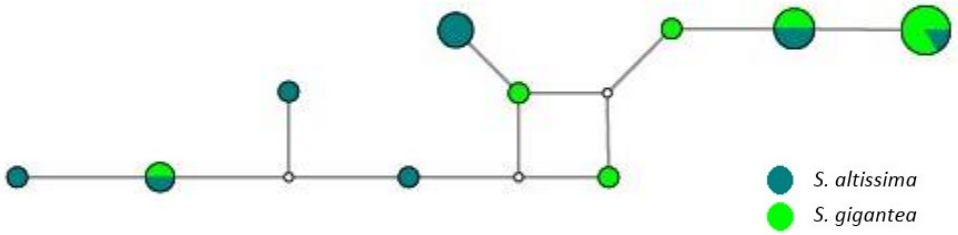

Figure 21. Haplotype network constructed by median joining of *Torymus capitis* COI sequences.

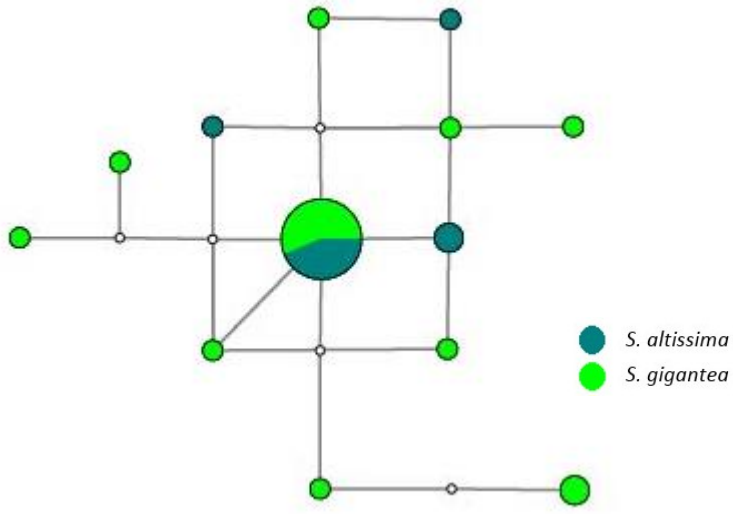

Figure 22: Haplotype network constructed by median joining of *Torymus capitis* ITS sequences.

f) Closterocerus solidaginis

A total of 22 ITS2 sequences were amplified in *Closterocerus solidaginis*. I was not able to amplify COI for this taxon and the resulting ITS sequences were relatively messy as well. No apparent structure is found by host in either the ML tree or the median-joining network (Figures 23 & 24). Three of the *Euthamia* samples clustered together with relatively low support, but another representative from this host plant was nested with the rest of the samples. It is possible that due to the relatively deep divergence seen of the *Euthamia* cluster that this group might be comprised of different species. AMOVA results suggest intermixing of the populations ($F_{st} = 0.17$) and no significant contribution to genetic variation by either host or site was detected (Table 8).

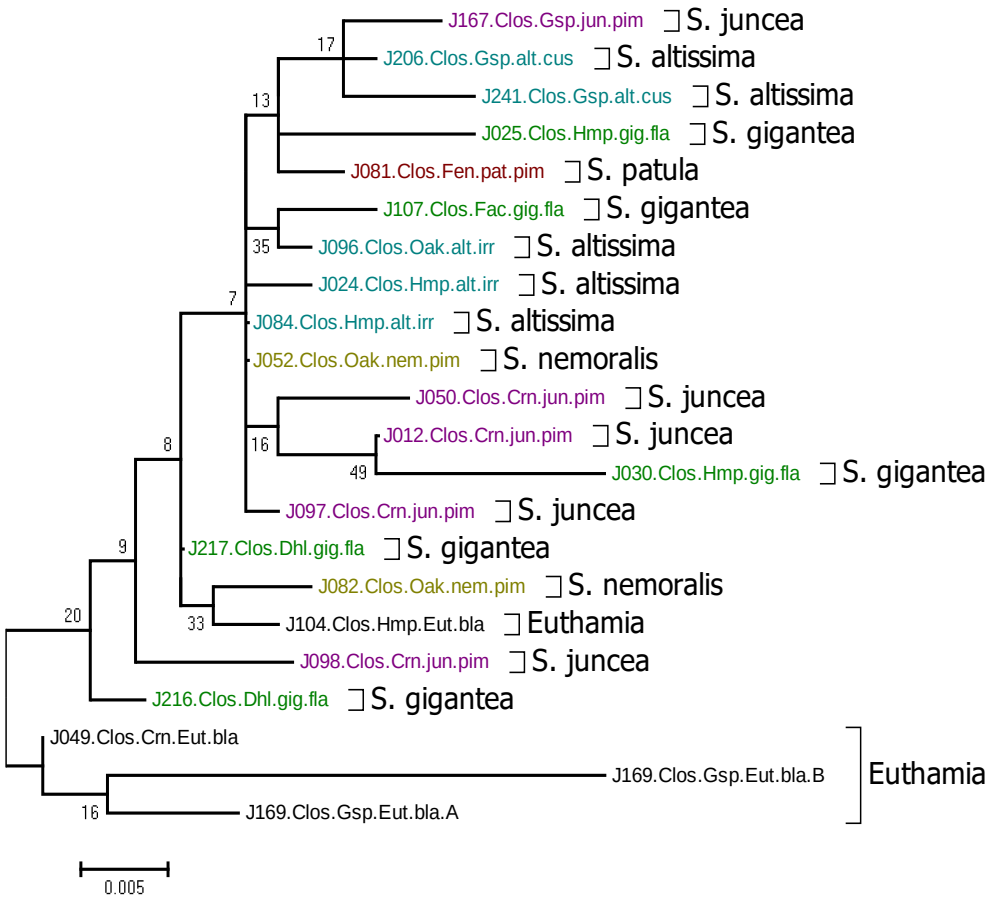

Figure 23. Maximum likelihood subtree of *Closterocerus solidaginis* for the ITS gene using 1000 bootstraps. All taxon code labels and branch colors are the same as Figure 3.

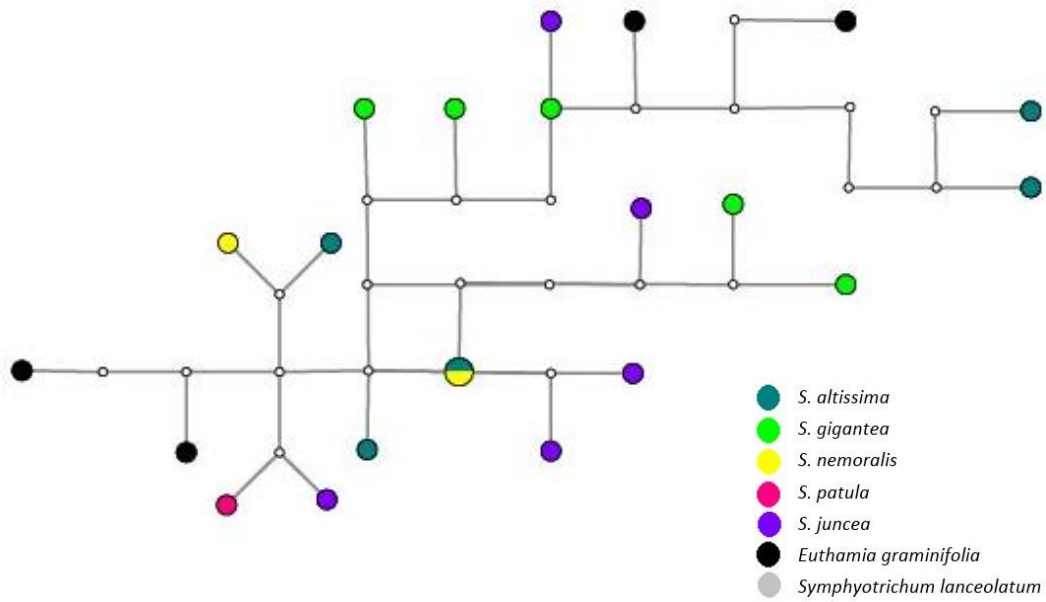

Figure 24. Haplotype network constructed by median joining of *Closterocerus solidaginis* ITS sequences.

Testing the Correlation between Parasitoid and Host Genetic Distances

Genetic distances among *Baryscapus fumipennis* populations had the strongest correlation with both *Asteromyia* host genetic distances and host plant (Figures 24 & 25). The mantel test revealed significant correlation coefficients of 0.919 with *Asteromyia* host distances and a 0.925 correlation with host plants (Table 9). None of the other parasitoid species exhibited a strong correlation with either *Asteromyia* host distances or plant distances (see Table 9).

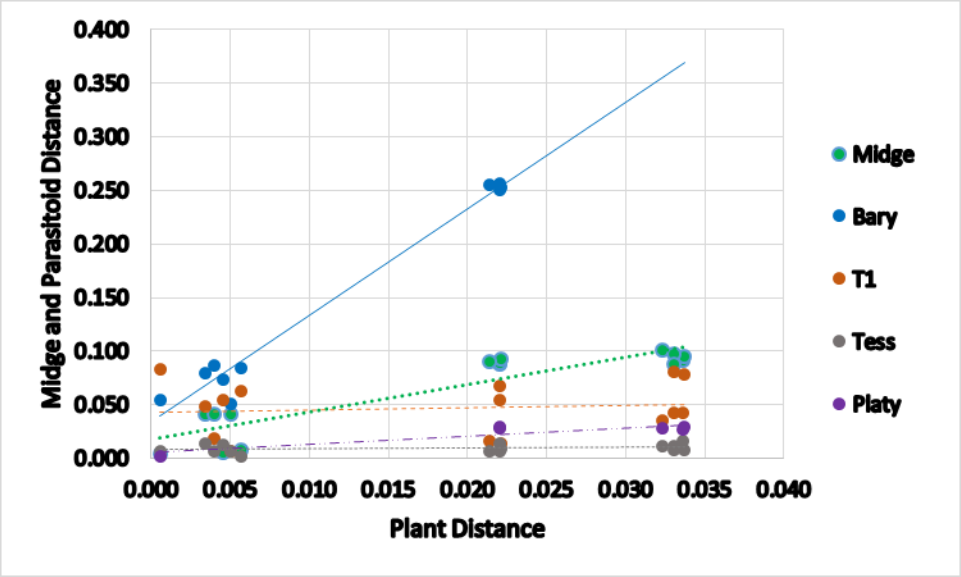

Figure 25. Mean pairwise genetic distances of midges and parasitoids plotted against plant distances.

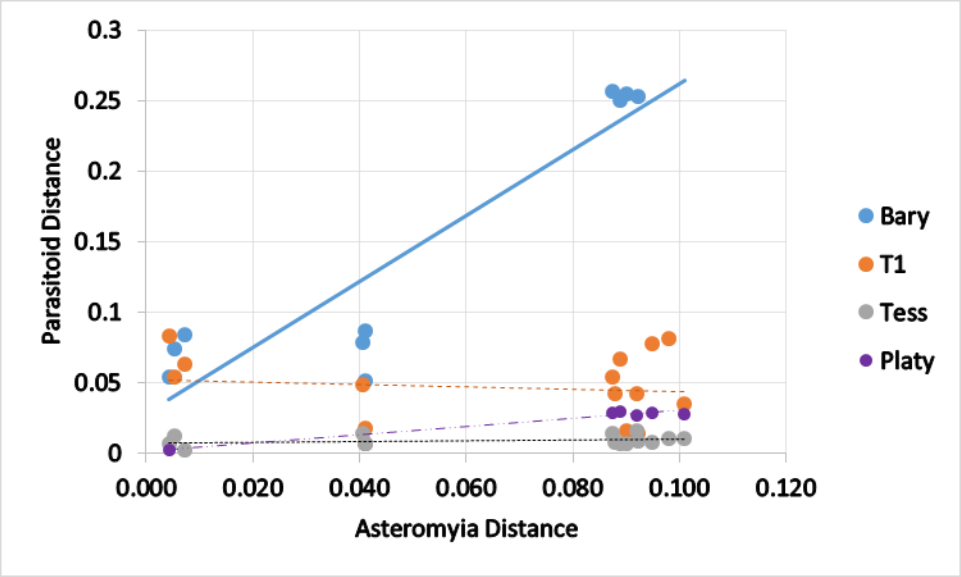

Figure 26. Mean pairwise genetic distances of parasitoids plotted against *Asteromyia* host distances.

Table 9. A Mantel test comparing the linear correlation between the matrix of insect host (*Asteroymyia*) and host plants with parasitoid phylogenetic distance matrices. Significant correlations are bolded and include an asterisk ($P < 0.05$).

Mantel Test Results: r(AB)

Parasitoids

	Baryscapus	***Platygaster***	***A. "T1"***	***A. tesserus***	***Closterocerus***
Host	**0.919***	0.520	-0.409	0.219	0.781
Host Plant	**0.925***	0.325	0.119	0.304	0.775

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DISCUSSION

Evidence of cascading host-associated genetic structure

Ecological and genetic isolation can occur as a result of adaptation to different hosts, facilitating host-associated divergence. Evidence of host-associated divergence is well documented in many phytophagous insects, including *Rhagoletis* fruit flies, leaf beetles, pea aphids, and *Eurosta* gall flies (Feder, 1998; Funk, 1998; Via, 1999; Craig et al., 1993). It is not known how frequently these divergence events cascade to the next trophic level or beyond, but it may be an important mechanism for generating the rich diversity seen in hymenopteran parasitoids. New studies are finding additional evidence that parasitoids may undergo host-associated differentiation in a cascading process, including two species of cynipid gall wasps, *Belonocnema treatae* and *Disholcaspis quercusvirens* (Egan et al., 2013) and the braconid parasitoid community that parasitizes *Rhagoletis* fruit flies (Hood et al., 2015).

There is strong genetic and ecological evidence that *Asteromyia* gall midges are undergoing host-associated differentiation as a consequence of their interactions with their goldenrod (*Solidago)* host plants, resulting in genetically distinct host-associated lineages (Stireman, et al., 2005; 2006; 2012). *Asteromyia* diversification has been facilitated by ecological opportunity; where they have exploited dozens of closely-related host plants in the highly diverse *Solidago* genus and Astereae tribe (Stireman et al., 2005; 2012). Similarly, parasitoids have a very intimate relationship with their host insects, so the parasitoids of *Asteroymia* may be undergoing host-associated divergence in sympatry as well in a cascading process (Stireman et al., 2006).

I tested whether parasitoids displayed evidence of host-associated genetic structure through the existence of genetically distinct host plant-associated structure as has been observed in their *Asteromyia* hosts (see Stireman et al., 2006). Three of the six parasitoid species examined in this study displayed evidence of host-associated genetic structure for at least one gene (COI) based on reconstructed phylogenies and haplotype networks. I found a wide range of results in a gradient-like fashion from strong genetic structure within host plant species in *Baryscapus fumipennis*, strong structure but limited sample size in *Aprostocetus sp. "T1"*, moderate structure related to host plant genera in *Platygaster solidaginis*, and weak to no structure in *Aprostocetus tesserus*, *Torymus captitis,* and *Closterocerus solidaginis*.

Baryscapus fumipennis in particular displayed strong host-plant associated structure in COI and modest structure at the ITS2 locus in ML trees and networks. The observed host-associated structure was statistically supported in the AMOVA analyses as well. Interestingly, this structure was detected within the *Solidago* genus, as this species was not found on other host plant genera, suggesting extremely fine host-association with either host-associated midge lineages or natal host plant. A very high F_{st} for COI and a similar correlation with host plant distances as their *Asteromyia* hosts (see fig. 25) suggests that this species is in the process of forming incipient species. Furthermore, the strong correlation with plant genetic distances suggests that *Baryscapus fumipennis* might have been diverging with *Solidago* host plants before *Asteromyia carbonifera* even colonized them. Perhaps *Baryscapus fumipennis* was utilizing a different host before shifting to *Asteromyia carbonifera* upon their radiation on *Solidago.*

Aprostocetus sp. "T1" exhibited similar structure to *Baryscapus fumipennis* according to the phylogenetic reconstructions and visual examination of haplotypes. However, strong evidence was not found in AMOVAs, likely due to the confounding factor of site where geography and host plant variation were confounded due to a limited sample size from relatively few sites. This limited sampling also interferes with our interpretation of F_{st} for this taxon, which was quite high for both COI and ITS2. More samples will be needed, but evidence of widespread host-associated genetic structure is suggestive in this undescribed species.

Platygaster solidaginis also appeared to be differentiated across host plants, but this was only detected in for mtDNA (COI), possibly due to poorly resolved data at the ITS2 locus. This species did not show the same fine-scale, within-species divergence as *Baryscapus* and *A. sp. "T1*", but formed distinct clades in the phylogeny by host plant genera. The observed clustering of host plant genera was statistically supported in the AMOVA as well, where almost 75% of the genetic variation between populations was driven by natal host plant for COI and an F_{st} of 0.84. Although some of the parasitoids displayed local differentiation in sympatry, the populations as a whole did not group into distinct clusters across their geographical distributions nor was geography as an isolating factor strongly supported in AMOVA analyses for well-sampled taxa. Thus, parasitoids that exhibited host plant-associated genetic structure and high fixation indices are very likely host races or perhaps incipient species (Powell et al., 2014).

None of the other remaining three parasitoid species revealed clear host-associated structure. However, this may be due to methodological issues and/or the ecology of these remaining parasitoids. For example, the COI gene in *Closterocerus*

solidaginis could not be amplified, so interpretation was limited to the ITS2 gene region which displayed virtually no genetic structure. *Torymus capitis* was only found on two closely related host plants, *Solidago gigantea* and *Solidago altissima*, so host-associated structure might not be expected at such a fine scale on such a limited range of hosts. *Aprostocetus tesserus* showed no structure at either locus, but in all of these cases, it's possible that the molecular tools used in this study were not adequate for detecting divergence; alternatively, these wasps may have diverged so recently that there has been insufficient time to detect divergence.

Evidence of cascading diversification in the form of host-associated genetic structure might result from parasitoids following midges evolutionarily as they genetically diverge into different host-associated forms on alternate host plants, resulting in the formation of cryptic species in both the midges and their parasitoids. Additionally, the parasitoids may be diversifying with the host plants as they utilize the plant's volatiles to aid their search effort for a suitable host insect. In order to determine if the parasitoids' genetic structure was more closely correlated with host plant or midge divergence, I compared mean genetic distance matrices of plants and the host-associated lineages of the midges and parasitoids. *Baryscapus fumpennis* was the only species that showed a significant correlation with both host plant and host insect genetic distances, although *Platygaster solidagnis* showed a modest correlation with host insect (Table 9). Unfortunately, it is not possible to accurately discern which source *Baryscapus* is actually diverging with as a result of these very high correlations with both host and host plant. *Asteromyia* midge lineages exhibit a near-perfect match with their natal host plant distances (see Stireman et al., 2005), so teasing the two apart as a driving factor on

parasitoid diversification is not possible at this time. However, high F_{st} values for both loci suggests that *Baryscapus* might have been diverging with host plants before the midges colonized them. It is possible that this effect is the result of parasitoid populations having lower effective population sizes than the midges where drift is acting to rapidly fix alleles. Alternatively, perhaps this species is a host plant generalist that has been using host plant-associated cues to locate suitable hosts and were able to successfully exploit *Asteromyia carbonifera* as a host range expansion. We do not know the extent to which different midge species share parasitoids, and this will need to be further investigated to draw accurate conclusions.

Behavioral responses to host plant volatiles

Parasitoid wasps must locate suitable hosts in an exceedingly complex environment during their short lives, so stimuli from their host or habitat that aid in host-searching are likely under strong selection. Since host-searching behavior in these wasps might be accomplished by utilizing different host plant volatiles in the environment, it is possible that reproductive isolation can proceed in wasp populations that respond to different host plant-associated cues. Such isolation arising from host plant preferences may reduce dispersal between populations on other plants, and may facilitate adaptive divergence in these parasitoids as seen in other examples of host-associated divergence in phytophagous insects (Egan et al., 2013; Powell et al., 2014).

A particularly interesting aspect of this multitrophic system is the galling behavior of the parasitoid's hosts. The fungal gall structure is completely stationary on the leaf of the plant as the midge eggs and larvae develop inside. This type of situation may benefit

the parasitoid's ability to find suitable hosts, because they can lock on to host plant-associated cues as a preliminary search effort. Then, once on the plant, it is likely that they use other cues (olfactory, visual, or perhaps tactile) to locate the galls. This may also encourage genetic isolation by host plant and might explain why the underlying genetic structure is so strongly correlated with natal plants in the phylogeny for some species. In this study, I found strong evidence that *Asteromyia* parasitoids exhibit a preference for natal host odors when tested in a 4-choice olfactometer; however, most of these natal host choices were made when different host genera were the alternative choices (i.e. *Solidago* vs. *Euthamia*) and further within-genus testing will be needed to determine parasitoid fidelity to natal host species' odors. Female parasitoids showed a much stronger preference for natal host plant odors than their male counterparts, particularly in *Platygaster solidaginis*, *Aprostocetus sp. "T1"*, and *Aprostocetus tesserus*. These findings are consistent with previous research in *Rhagoletis pomonella* parasitoids, where female wasps (*Diachasma alloeum*) were found to orient toward natal fruit volatiles in a Y-tube olfactometer but were antagonized by non-natal volatiles (Forbes et al., 2009).

Female parasitoids likely exhibit a stronger response to olfactory cues from natal host plants due to their need to efficiently find a suitable host in an environment littered with both relevant and irrelevant stimuli. It is thought that traces of plant affiliated chemical cues are carried through adult emergence during development in the gall, which might explain the preferential responses of adults to their natal host plants (Graziosi & Rieske, 2013). Since the female would have emerged from a suitable host insect on that particular natal plant, perhaps she is relying on these same plant cues to aid her own host searching efforts. However, males might also benefit by preferentially responding to natal

host plant volatiles as it might offer an opportunity to find females to mate with as a consequence of their own searching behavior, and this may further reproductive isolation. Larger sample sizes of male parasitoids will be needed to confirm the observed lack of a behavioral response to natal host plant cues since they were the under-represented sex in this study (N = 12).

The strong behavioral responses of both *Platygaster solidaginis* and *Aprostocetus sp. "T1"* reflect the underlying host-associated genetic structure seen in the phylogenetic evidence very well. However, despite substantial evidence of host-associated divergence in *Baryscapus fumipennis*, a preference for natal host odors was not observed in females of this species. Additional olfactometry trials may be needed for this species in order to determine whether or not they are attracted to natal host odors. Interestingly, *Aprostocetus tesserus* exhibited a very strong preference for natal host odors, but showed virtually no genetic differentiation. As noted earlier, it is possible that this species has diverged so recently that we are unable to detect any accumulation of genetic differences between populations, or perhaps the molecular tools used in this study were not powerful enough to detect divergence. Fine-scale allele frequency data over multiple loci may be needed to further investigate the potential for host-associated genetic structure in *Aprostocetus tesserus* and the other parasitoids.

Utilizing host plant odors to locate hosts and being very sensitive to these cues might promote isolation, but we do not know how this varies across each of these species. Some of the behavioral data suggests biased responses that correspond with genetic structure as predicted, but perhaps those species that did not show genetic structure do not use host plant cues. Traits that may promote or inhibit the formation of distinct

genetic populations might include: (i) the use of other cues that are not dependent on host plant; (ii) dispersal ability, where low dispersal rates might promote genetic structure as a consequence of mating in isolated habitat patches; (iii) temporal isolation resulting from differing host phenology; (iv) reproductive potential in pro-ovigenic vs. synovigenic parasitoids, where the prior eclose with all of their eggs and the latter must feed to produce more eggs. This might allow for more generalized attacks by pro-ovigenic species who may dump all of their eggs into a host, while synovigenic species may afford to be more selective due to their more limited egg production. (v) The biology of the parasitoid, where some are endoparasitoids and others are ectoparasitoids. One might expect that endoparasitoids (e.g., *Platygaster solidaginis*) are more likely to specialize since they must overcome the host's physiological defenses while ectoparasitoids (e.g., *Torymus capitis*) might be able to use a more general host range. This is observed in the biology of *Torymus* which is not only an ectoparasitoid, but also a facultative hyperparasitoid that probably uses other cues such as visual stimuli to find hosts as found in *Torymus sinensis* by Graziosi and Rieske (2013).

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CONCLUSION

This study has revealed that parasitoids of *Asteromyia* gall midges exhibit idiosyncratic patterns of divergence in relation to plant and host divergence. The interactions between plants and insects in this multitrophic system may have contributed to the diversification of not only the plant-feeding midges, but also their natural wasp enemies in the form of a positive feedback loop. As midges colonize novel host plants, they can form plant-specific host-associated lineages. These initial shifts to new hosts by the herbivore may actually be a consequence of avoiding their natural enemies ("enemy free space"), and in turn, parasitoids may follow suit as they adapt to divergent selective pressures on these novel hosts and form their own host-associated lineages on the midge races or natal host plants (Godfray, 1994; Heard et al., 2013). These interactions may create a constant cycle of diversification between parasitoids and their phytophagous hosts, providing a mechanism for the rich diversity observed in these insect groups.

Further genetic and behavioral sampling will be needed to confirm these findings in under-sampled taxa from this study, and investigation of multitrophic interactions in other systems might provide further insight into just how common host-associated divergence cascades into higher trophic levels. With advances in high-throughput genome sequencing, large data sets can be examined to better understand the underlying processes that promote population divergence (Feder et al., 2012). Additionally, advanced tracking software in combination with more suitable environmental conditions might provide additional empirical support to the behavioral responses of parasitoids and

their hosts by mapping preferences and orientation through time (i.e. LoliTrack software by Loligo Systems).

LITERATURE CITED

Abrahamson, G. W., Sattler, J. F., McCrea, K. D., & Weis, A. E. 1989. Variation in Selection Pressures on the Goldenrod Gall Fly and the Competitive Interactions of Its Natural Enemies. *Oecologia*. 79(1): 15-22.

Abrahamson, G. W. & Blair, P. C. 2008. Sequential Radiation Through Host-Race Formation: Herbivore Diversity Leads to Diversity in Natural Enemies. In: K. Tilmon, ed. Specialization, Speciation, and Radiation: the Evolutionary Biology of Herbivorous Insects. University of California Press, USA, pp. 188-202.

Bailey R, Schönrogge K, Cook JM, Melika G, Csóka G, et al. 2009. Host Niches and Defensive Extended Phenotypes Structure Parasitoid Wasp Communities. PLoS Biol 7(8).

Borkent, A. & Bissett, J. 1985. Gall midges (Diptera: Cecidomyiidae) are vectors for their fungal symbionts. *Symbiosis*. 1(2): 185-194.

Cain, M.L., Carson, W. P., and Root, R. B. 1991. Long-term suppression of insect herbivores increases the production and growth of *Solidago altissima* rhizomes. *Oecologia* 88:251-257.

Craig, T. P., Itami, J. K., Abrahamson, W. G., & Homer J. D. 1993. Behavioral evidence for host-race formation in Eurosta solidaginis. *Evolution*; 47: 1696-1710.

Danforth, B.N., Sauquet, H. & Packer, L. (1999) Phylogeny of the bee genus *Halicus* (Hymenoptera: Halictidae) based on parsimony and likelihood analyses of nuclear EF-1α sequence data. *Molecular Phylogenetics and Evolution*, 13, 605–618.

Duffy, J. E. 2009. Why biodiversity is important to the functioning of real-world ecosystems. *Front Ecol Environ*; 7(8): 437–444.

Dorchin, N., Joy, J. B., Hilke, L. K., Wise, M. J. and Abrahamson, W. G. 2015. Taxonomy and phylogeny of the *Asphondylia* species (Diptera: Cecidomyiidae) of North American goldenrods: challenging morphology, complex host associations, and cryptic speciation. *Zoological Journal of the Linnean Society,* 174: 265–304.

Excoffier L, Laval G, Schneider S. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinfor-matics Online 1: 47–50.

Farrell, B. D. 1998. 'Inordinate fondness' explained: why are there so many beetles? *Science*. 281:555-559.

Feder, J. L. 1998. The apple maggot fly, *Rhagoletis pomonella*: flies in the face of conventional wisdom about speciation? Howard, D. J. & Berlocher, S. H. (Eds.) Endless Forms: Species and Speciation. New York, Oxford University Press.

Feder J. L., Egan S. P., Forbes A. A. 2012. Ecological Adaptation and Speciation: The Evolutionary Significance of Habitat Avoidance as a Postzygotic Reproductive Barrier to Gene Flow. *International Journal of Ecology*. 2012: 15 pages.

Forbes, A. A., Powell, H. T., Stelinski, L. L., Smith, J. J. & Feder, L. J. 2009. Sequential Sympatric Speciation Across Tropic Levels. *Science*; 323, 776-779.

Funk, D. J. 1998. Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. *Evolution*; 52: 1744-1759.

Girling, R. D., Stewart-Jones, A., Dherbecourt, J., Staley, J. T., Wright, D. J., & Poppy, G. M. 2011. Parasitoids select plants more heavily infested with their caterpillar hosts: a new approach to aid interpretation of plant headspace volatiles. *Proceedings of the Royal Society*. 278(1718), 2646–53.

Gagné R. J. 1968. A taxonomic revision of the genus *Asteromyia* (Diptera: Cecidomyiidae). *Misc. Pubs. Entomol. Soc. Am.* 6: 1-40.

Godfray, H. C. J. 1994. Parasitoids: Behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ, USA.

Graziosi, I., & Rieski, L. 2013. Response of *Torymus sinensis*, a parasitoid of the gallforming *Dryocosmus kuriphilus*, to olfactory and visual cues. *Biological Control*. (67): 137-142.

Hall, Barry. 2013. Building Phylogenetic Trees with Molecular Data with MEGA. *Mol. Biol. Evol.* 30(5): 1229–1235.

Heath, J. & Stireman, J. O. III. 2010. Dissecting the association between a gall midge, *Asteromyia carbonifera*, and its symbiotic fungus, *Botryosphaeria dothidea*. *Entomologia Experimentalis et Applicata.* (137): 36- 49.

Heard, S. B., Stireman, J. O. III., Nason, J. D., Cox, G. H., Kolacz, C. R., & Brown, J. M. 2013. On the elusiveness of enemy-free space : spatial, temporal , and host-plant-related variation in parasitoid attack rates on three gallmakers of goldenrods.

Heraty, John M. et al. 2013. A phylogenetic analysis of the megadiverse Chalcidoidea (Hymenoptera). *Cladistics*. 29(5): pages 466–542.

Hood, G. R., Forbes, A. A., Powell, T. H., Egan, S. P., Hamerlinck, G., Smith, J. J., & Feder, J. L. 2015. Sequential divergence and the multiplicative origin of community diversity. PNAS; 114(44).

Hurst, G. D., & Jiggins, F. M. 2005. Problems with mitochondrial DNA as a marker in population phylogeographic and phylogenetic studies: the effects of inherited symbionts *Proc. R. Soc. B*. (272) 1525–1534.

Jeder, L. J., & Forbes, A. A. 2010. Sequential speciation and the diversity of parasitic insects. *Ecological Entomology*; 35(1), 67-76.

Laureto, P. J., & Barkman, T. J. 2011. Nuclear and chloroplast DNA suggest a complex single origin of the threatened allopolyploid S*olidago houghtonii* (Asteraceae) invlovling reticulate evolution and introgression. *Syst. Bot*. 36: 209-226.

Li, Y., Zhou, X., Feng, G., Hu, H., Niu, L., Herbert, P. D. N., & Huang, D. 2010. COI and ITS2 sequences delimit species, reveal cryptic taxa and host specificity of fig-associated Sycophila (Hymenoptera, Eurytomidae). *Molecular Ecology Resources*; 10(1), 31–40.

Librado, P. & Rozas, J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*; 25(11):1451-1452.

McLeish, M. J., Beukman, G., van Noort, S., & Wossler, T. C. 2012. Host plant species conservatism and ecology of a parasitoid fig wasp genus (Chalcidoidea; Sycoryctinae; Arachonia). *PloS One*, *7*(9), e44804.

Millar, N. 2001. Biology statistics made simple using Excel. *School Science Review*. 83(303).

Nambiar-Veetil, M., Sangeetha, M., Rani, K. S., Aravinthakumar, V., Selvakesavan, R. K., Balasubramanian, A., Venkatachalam, R., Mary, A. S., Prasanth, J., & Kumar, K. 2011. Identification of insect-specific target genes for development of RNAi based control of the Eucalyptus gall pest Leptocybe invasa Fisher & La Salle (Hymenoptera: Eulophidae). *BMC Proceedings*; 5(Suppl 7):P98.

Novotny, V., Basset, Y., Miller, S. E., Weiblen, G. D., Bremer, B., Cizek, L. & Drozd, P. 2002. Low host specificity of herbivorous insects in a tropical forest. *Nature*. 416, 841–844.

Poelman, E. H., van Loon, J., Dicke, M. 2008. Consequences of variation in plant defense for biodiversity at higher trophic levels. *Trends in plant science*. 13(10): 534-541.

Posada, D., Buckley, T. R. 2004. Model selection and model averaging in phylogenetics: advantages of akaike information criterion and bayesian approaches over likelihood ratio tests. *Systematic Biology*. 53(5):793-808.

Powell, T. H., Forbes, A. A., Hood, G. R., & Feder, J. L. 2014. Ecological adaptation and reproductive isolation in sympatry: genetic and phenotypic evidence for native host races of *Rhagoletis pomonella*. *Mol. Ecol*. 23, 688-704.

Root, R. B. 1996. Herbivore pressure on goldenrods (*Solidago altissima*): its variation and cumulative effects. Ecology 77:1074-1087.

Schilling, E. E., Beck, B. J., Calie, J. P., & Small, R. L. 2008. Molecular Analysis of Solidaster CV. Lemore, A Hybrid Goldenrod (Asteraceae). *J. Bot. Res. Inst. Texas;* 2(1): 7-18. 2008.

Schluter, D. 2001. Ecology and the origin of species. *Trends in Ecology and Evolution*. 16(7):372-380.

Semple J. C. & Cook, R. E. 2003. *Flora of North America*. Vol. 20, pp. 3,12, 14, 15, 18, 19, 97, 102, 105, 106, 107.

Silva, J. W. P., Bento, J. M. S. & Zucchi, R. A. 2007. Olfactory response of three parasitoid species (Hymenoptera: Braconidae) to volatiles of guavas infested or not with fruit fly larvae (Diptera: Tephritidae). *Biological Control*. (41): 304-311.

Stephens, M. & Donnelly, P. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am. J. Hum. Genet*., 73, 1162–1169.

Stireman, J.O. III, Nason, J. D. and Heard, S. 2005. Host-associated genetic differentiation in phytophagous insects: General phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. *Evolution*; 59:2573-2587.

Stireman, John O., Nason, N., D., Heard, B. S., Seehawer, M., J. 2006. Cascading host-associated genetic differentiation in parasitoids of phytophagous insects. *Proceedings of the Royal Society*; 273, 523-530.

Stireman, J. O. III, Janson, E. M., Carr T. G., Devlin H. & Abbot P. 2008. Evolutionary Radiation of *Asteromyia carbonifera* (Diptera: Cecidomyiidae) gall morphotypes

on the goldenrod *Solidago altissima* (Asteraceae). *Biological Journal of the Linnean Society*. (95): 840-858.

Stireman, J. O. III, Devlin, H., Carr T. & Abbot P. 2010. Evolutionary diversification of the gall midge genus Asteromyia (Cecidomyiidae) in a multitrophic ecological context. *Molecular Phylogenetics and Evolution*. 54(1): 194- 210.

Stireman, J. O. III, Devlin, H., & Abbot, P. 2012. Rampant host- and defensive phenotype-associated diversification in a goldenrod gall midge. *Journal of Evolutionary Biology*.(25) 1991-2004.

Taekul, C., Valerio, A. A., Austin, A. D., Klompen, H., & Johnson, N. 2013. Molecular phylogeny of telenomine egg parasitoids (Hymenoptera: Platygastridae s.I.: Telenominae):evolution of host shifts and implications for classification. *Systematic Entomology*, 39: 24-35.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28: 2731-2739.

Tentelier, C. and Fauvergue, X. 2007. Herbivore-induced plant volatiles as cues for habitat assessment by a foraging parasitoid. *Journal of Animal Ecology*. 76: 1-8.

Tooker, J. F., Rohr, J. R., Abrahamson, W. G., & De Moraes, C. M. 2008. Gall insects can avoid and alter indirect plant defenses. *The New Phytologist*, *178*(3), 657–71.

Turlings, C. J., Davison, A. C. & Tamo, C. 2004. A six-arm olfactometer permitting simultaneous observation of insect attraction and odour trapping. *Physiological Entomology.* (29):25- 55.

Vet, L. E. M., Van Lenteren, J. C., Heymans, M. & Meelis, E. 1983. An airflow olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. Physiological Entomology. (8): 97- 106.

Via, S. 1999. Reproductive isolation between sympatric races of pea aphids. I: Gene flow restriction and habitat choice. *Evolution*; 53: 1446-1457.

Weis, A. E. 1982a. Use of Symbiotic Fungus by the Gall Maker *Asteromyia carbonifera* to Inhibit Attack by the Parasitoid *Torymus capite*. *Ecological Society of America*. Vol. 63(5): 1602-1605.

Weis, A. E. 1982b. Resource utilization patterns in a community of gall-attacking parasitoids. *Environmental Entomology*. (11): 809-815.

Weis, A. E. 1983. Patterns of parasitism by *Torymus capite* on hosts distributed in small patches. *Journal of Animal Ecology*. (52): 867-877.

Weis, A. E. & Abrahamson, W. G. 1986. Evolution of host-plant manipulation by gall makers: ecological and genetic factors in the *Solidago-Eurosta* system. *The American Naturalist*. 127(5): 681-695.

Ya-Jie, J., De-Xing, Z., & Li-Jun, H. 2003. Evolutionary conservation and versatility of a new set of primers for amplyifying the ribosomal internal transcribed spacer regions in insects and other invertebrates. *Molecular Ecology Notes*. (3): 681-695.

APPENDIX I

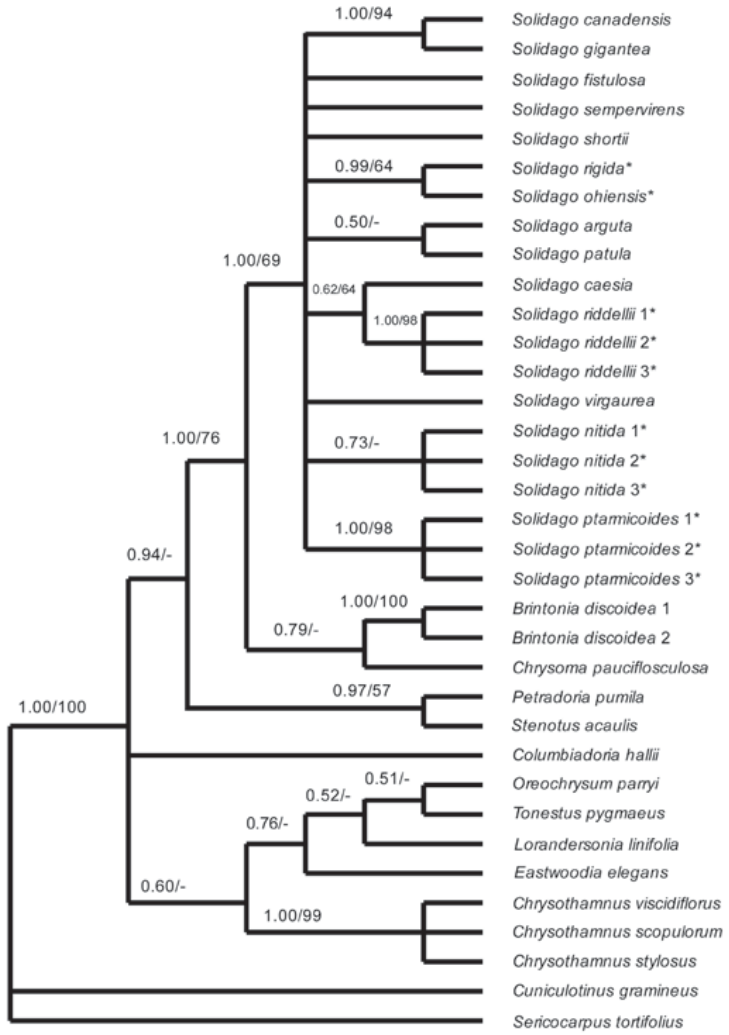

Figure 27. Phylogenetic relationships among *Solidago* species, adapted from Schilling et al., 2008. Note that *Solidago canadensis* and *Solidago altissima* are the same species. Phylogenetic placement of *Solidago ptarmicoides* relative to *Solidago* and related genera, based on combined analysis of ITS and ETS data. Shown is the tree produced by Bayesian analysis (the strict consensus of 408 minimum length trees in a single island,

CI=0.69, RI=0.85, obtained from parsimony analysis was topologically similar although with less resolution of clades), using *Cuniculotinus* and *Sericocarpus* as outgroups. Support values from Bayesian and bootstrap (if greater than 50%) shown above branches. Asterisks designate species of *Solidago* sect. Ptarmicoidea (Oligoneuron).

Tables 10-18. Contingency tables and binomial probabilities of olfactory responses by parasitoids that were under-sampled or failed to produce a significant test result**.**

Table 10. Behavioral responses of male *Aprostocetus sp. "T1"*.

A. sp. 'T1' **(Male) - Choices and Host Plant Origin**			
Host Plant	Natal Choice	Other Choice	Binomial Probability (PX=x)
alt	1	0	-
gig	0	1	-
jun	0	0	-
Eut	0	0	-
Sym	0	0	-
Total	1	1	**0.375**

Table 11. Behavioral responses of male *Aprostocetus tesserus.*

A. tesserus **(Male) - Choices and Host Plant Origin**			
Host Plant	Natal Choice	Other Choice	Binomial Probability (PX=x)
alt	0	0	-
gig	0	1	-
jun	2	1	-
Eut	0	0	-
Sym	0	0	-
Total	2	2	**0.21093**

Table 12. Behavioral responses of male *Platygaster solidaginis.*

Platygaster solidaginis **(Male) - Choices and Host Plant Origin**			
Host Plant	Natal Choice	Other Choice	Binomial Probability (PX=x)
alt	0	0	-
gig	0	0	-
jun	0	1	-
Eut	0	0	-
Sym	1	0	-
Total	1	1	**0.375**

Table 13. Behavioral responses of female *Torymus capitis.*

Torymus capitis **(Female) - Choices and Host Plant Origin**			
Host Plant	Natal Choice	Other Choice	Binomial Probability (PX=x)
alt	1	0	-
gig	1	0	-
jun	0	0	-
Eut	0	0	-
Sym	0	0	-
Total	2	0	**0.0625**

Table 14. Behavioral responses of male *Torymus capitis.*

Torymus capitis **(Male) - Choices and Host Plant Origin**			
Host Plant	Natal Choice	Other Choice	Binomial Probability (PX=x)
alt	0	0	-
gig	1	1	-
jun	0	0	-
Eut	0	0	-
Sym	0	0	-
Total	1	1	**0.375**

Table 15. Behavioral responses of female *Closterocerus solidaginis.*

Closterocerus solidaginis **(Female) - Choices and Host Plant Origin**			
Host Plant	Natal Choice	Other Choice	Binomial Probability (PX=x)
alt	0	0	-
gig	0	1	-
jun	0	0	-
Eut	2	1	-
Sym	1	0	-
Total	3	2	**0.08789**

Table 16. Behavioral responses of male *Closterocerus solidaginis.*

Closterocerus solidaginis **(Male) - Choices and Host Plant Origin**			
Host Plant	Natal Choice	Other Choice	Binomial Probability (PX=x)
alt	0	1	-
gig	0	0	-
jun	0	1	-
Eut	0	0	-
Sym	0	0	-
Total	0	2	**0.5625**

Table 17. Behavioral responses of female *Baryscapus fumipennis*.

Baryscapus fumipennis (Female) - Choices and Host Plant Origin			
Host Plant	Natal Choice	Other Choice	Binomial Probability (PX=x)
alt	1	2	-
gig	0	3	-
jun	0	0	-
Eut	0	0	-
Sym	0	0	-
Total	1	5	**0.395507**

Tables 18-24. Mean pairwise genetic distance matrices.

Table 18. The mean pairwise distances (COI) between natal host lineages of *Asteromyia carbonifera*.

***Asteroymyia carbonifera* - COI**

Host Plant	Sym	Eut	S. gig	S. alt	S. nem	S. pat
Sym	-					
Eut	0.000	-				
S. gig	0.000	0.088	-			
S. alt	0.000	0.089	0.004	-		
S. nem	0.000	0.092	0.041	0.041	-	
S. pat	0.000	0.090	0.005	0.007	0.041	-

Table 19. The mean pairwise distances (COI) between host plants (*Solidago* and Astereae).

Host Plants - COI

Host Plant	Sym	Eut	S. gig	S. alt	S. nem	S. pat
Sym	-					
Eut	0.032	-				
S. gig	0.034	0.022	-			
S. alt	0.034	0.022	0.001	-		
S. nem	0.033	0.022	0.003	0.004	-	
S. pat	0.033	0.021	0.005	0.006	0.005	-

Table 20. The mean pairwise distances (COI + ITS2) between natal host populations of *Baryscapus fumipennis.*

***Baryscapus fumipennis* - COI and ITS**

Host Plant	Eut	S. gig	S. alt	S. nem	S. pat
Eut	-				
S. gig	0.257	-			
S. alt	0.250	0.054	-		
S. nem	0.253	0.079	0.087	-	
S. pat	0.255	0.074	0.084	0.051	-

Table 21. The mean pairwise distances (COI + ITS2) between natal host populations of *Aprostocetus sp. 'T1'*.

***Aprostocetus sp. 'T1'* - COI and ITS**

Host Plant	Sym	Eut	S. gig	S. alt	S. nem	S. pat
Sym	-					
Eut	0.035	-				
S. gig	0.042	0.054	-			
S. alt	0.078	0.067	0.083	-		
S. nem	0.081	0.014	0.049	0.018	-	
S. pat	0.042	0.016	0.054	0.063	0.007	-

Table 22. The mean pairwise distances (COI + ITS2) between natal host populations of *Platygaster solidaginis.*

***Platygaster solidaginis* - COI and ITS**

Host Plant	Sym	Eut	S. gig	S. alt	S. jun	S. pat
Sym	-					
Eut	0.105	-				
S. gig	0.197	0.134	-			
S. alt	0.150	0.098	0.137	-		
S. jun	0.182	0.132	0.177	0.132	-	
S. pat	0.301	0.255	0.356	0.303	0.255	-

Table 23. The mean pairwise distances (COI + ITS2) between natal host populations of *Aprostocetus tesserus.*

***Aprostocetus tesserus* - COI and ITS**

Host Plant	Sym	Eut	S. gig	S. alt	S. nem	S. pat
Sym	0.000					
Eut	0.011	0.000				
gig	0.016	0.014	0.000			
alt	0.008	0.007	0.007	0.000		
nem	0.011	0.009	0.014	0.007	0.000	
pat	0.008	0.007	0.012	0.002	0.007	0.000

Table 24. The mean pairwise distances (COI + ITS2) between natal host populations of *Closterocerus solidaginis.*

***Closterocerus solidaginis* - ITS**

Host Plant	Eut	S. gig	S. alt	S. pat
Eut	-			
S. gig	0.014	-		
S. alt	0.013	0.009	-	
S. pat	0.009	0.006	0.009	-